A STUDY OF POLYSOMNOGRAPHY AND SERUM GHRELIN LEVELS IN PATIENTS WITH GENERALIZED TONIC-CLONIC SEIZURES

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& EXPERIMENTAL MEDICINE,

MADRAS MEDICAL COLLEGE AND HOSPITAL,

CHENNAI –600003

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

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APRIL 2015

CERTIFICATE

This is to certify that the dissertation entitled "**Study of Polysomnography and serum ghrelin levels in patients with Generalized tonic-clonic seizures"** by the candidate Dr. K. MEENAKUMARI, for M.D Physiology is a bonafide record of the research done by her during the period of study (2012 –2015) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

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ABBREVIATION

S.NO	ABBREVIATION	EXPANSION
1.	AASM	American Academy of Sleep Medicine
2.	AHI	Apnea-Hypopnoea Index
3.	BMI	Body mass Index
4.	CSA	Central sleep Apnea
5.	DI	Desaturation Index
6.	ECG	Electrocardiogram
7.	EEG	Electroencephalogram
8.	EMG	Electromyogram
9.	EOG	Electro-oculogram
10.	LSAT	Lowest saturation of oxygen in blood
11.	NREM	Non rapid eye movement
12.	REM	Rapid Eye Movement

S.NO	ABBREVIATION	EXPANSION
13.	PSG	Polysomnography
14.	R&K Criteria	Rechtschaffen and A.Kales
15.	RDI	Respiratory disturbance Index
16.	REM	Rapid eye movement
17.	RERA	Respiratory effort related Arousal
18.	SDB	Sleep disordered breathing
19.	SWS	Slow wave sleep
20.	TRT	Total recording time
22.	TST	Total sleep time
23.	WASO	Wake after sleep onset
24.	IEDS	Interictal epileptiform discharges
25	GH	Growth hormone
26	AG	Acylated Ghrelin
27	UAG	Unacylated Ghrelin

S.NO	ABBREVIATION	EXPANSION
28	GTCS	Generalized tonic clonic seizures
29	AEDs	Antiepileptic drugs
30	PHT	Phenobarbitone
31	CBZ	Carbamazepine
32	GBP	Gabapentin
33	LTG	Lamotrigine

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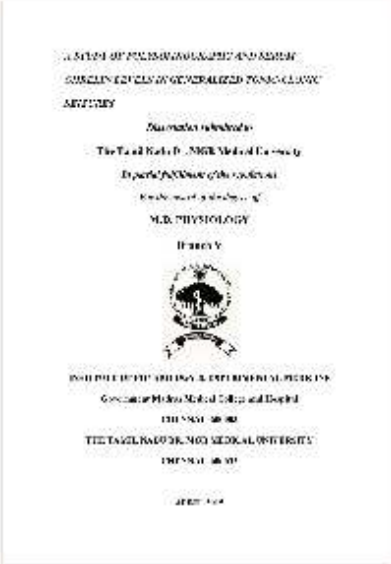

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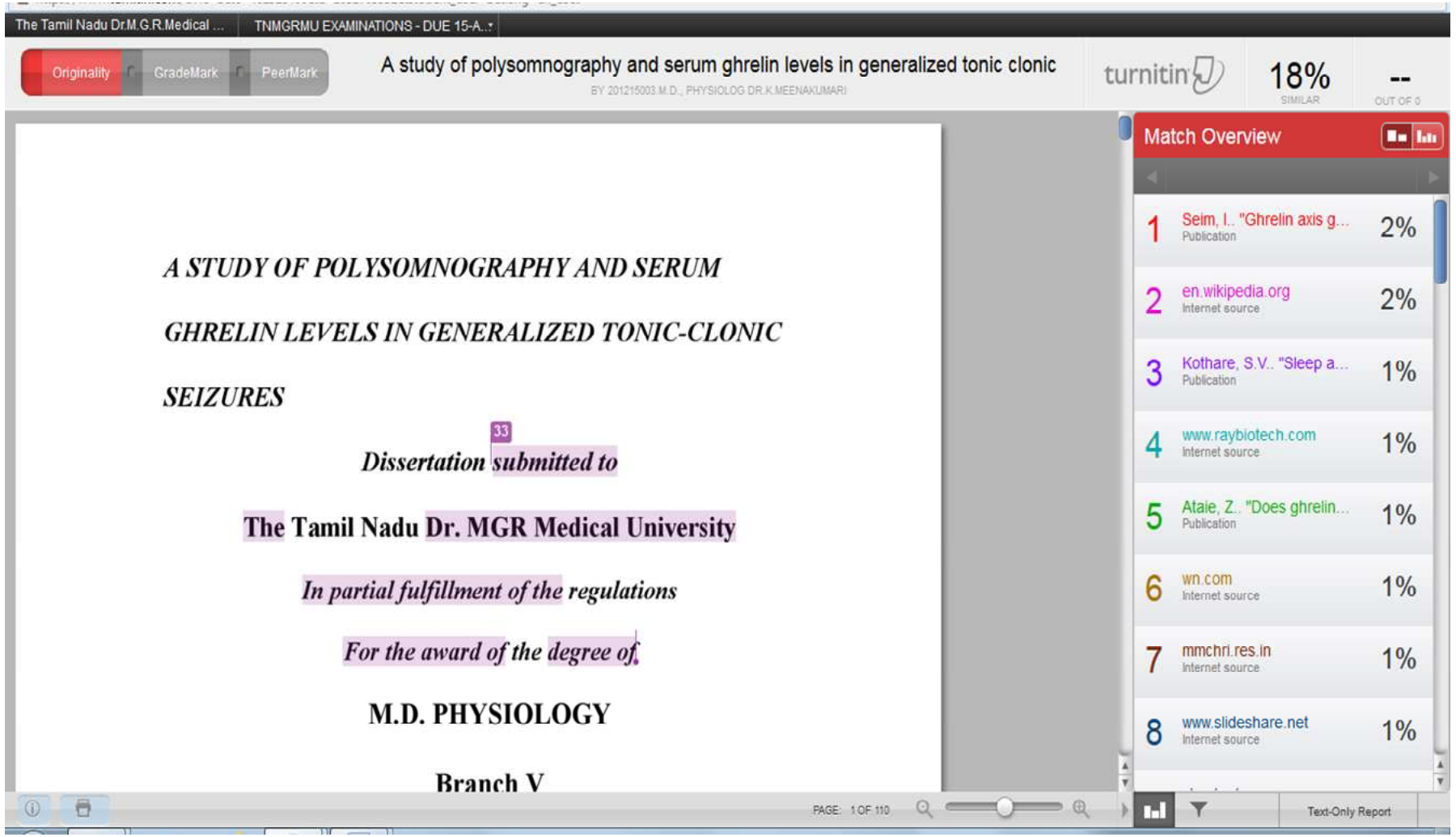
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ABSTRACT

A STUDY OF POLYSOMNOGRAPHY AND SERUM GHRELIN LEVELS IN PATIENTS WITH GENERALIZED TONIC CLONIC SEIZURES:

Degree for which submitted: Doctor of Medicine (MD) in Physiology

Supervisor and guide: Dr. K. PADMA

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Department: Institute of physiology and experimental medicine

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University: The Tamilnadu Dr. M .G. R .Medical university,

Chennai- 600032

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BACKGROUND:

There is a complex relationship between sleep and epilepsy. They are reciprocal to each other.

METHOD:

A total of 30 generalized tonic clonic seizure patients and 30 controls were subjected to polysomnography. It simultaneously records the three physiological measures that define the stages of muscle tone, recorded through Electromyogram (EMG), eye movements, recorded through the Electro-oculogram (EOG) and brain activity, recorded through the Electroencephalogram (EEG), and compared with equal number of normal individuals. Ghrelin is peptide hormone, secreted from stomach. Its level was measured in the fasting state. Its level increased before food intake and decreased after food intake.

RESULTS:

The mean age of the study groups were comparable 31.10±5.6 for GTCS patients and 30.03±6.26 for control groups as well as body mass index for GTCS patients (26.89±0.99) and for control groups (26.30±1.46). In this study, sleep architectural changes, mainly prolonged stage I (18.35±2.85 for GTCS and 14.6±2.6 f0r control group)and stage II of NREM sleep and reduced REM sleep(63.93±13.21 for GTCS and 77.27±13.3 for control group) occurs in GTCS patients than comparative with control group leads to fragmentation of sleep. And also serum ghrelin levels were decreased in these patients.

CONCLUSION:

Sleep disturbances are more common in the epileptic patients. It will further worsening the seizure propensity. Identifying and treating these associated sleep problems, also an important part in the management of epilepsy.

Keywords: ghrelin, generalized tonic clonic seizures, polysomnography.

A STUDY OF POLYSOMNOGRAPHY AND SERUM GHRELIN LEVELS IN PATIENTS WITH GENERALIZED TONIC CLONIC SEIZURES

INTRODUCTION:

Epilepsy is a chronic disorder characterized by a spontaneous tendency for recurrent seizures. Seizures are the clinical manifestation of abnormally hyper excitable cortical neurons.

The clinical manifestations of the attack may vary from complex abnormalities of behaviour including generalized or focal convulsions to momentary spells of impaired consciousness.

Based on the type of behaviour and brain activity, seizures are classified into two categories generalized and partial. Generalized seizures are produced by electrical impulses arise from overall brain. Partial seizures are produced by electrical impulses arise from a small area of brain.

The most common type is generalized tonic - clonic seizures or grandmal seizures.

The terms seizure, convulsion, or epilepsy are often associated with generalized tonic clonic seizures.

Generalized tonic- clonic seizures may occur in any age. They can occur as a single episode or as a part of a repeated, chronic illness.

Symptoms of Generalized tonic - clonic seizures:

Many persons with these seizures have vision, taste, smell, or sensory changes, hallucinations, or dizziness before the seizure. This is called an aura.

The seizures usually result in rigid muscles. This is followed by violent muscle contractions and loss of consciousness. Other symptoms are biting of the tongue, clenched teeth, loss of urine or stool control, difficulty in breathing, blue skin colour.

After the seizure, the person may have confusion, sleepiness that lasts for 1 hour, loss of memory, headache.

Risk factors for generalized tonic- clonic seizures:

A family history of seizure disorder, any injury to the brain from trauma, stroke, previous infection and other causes, sleep deprivation, electrolyte imbalance, drug abuse, heavy alcohol use.

The incidence of sudden death is 24 times higher in patients with epilepsy than in general population.

Risk factors for sudden death in epilepsy (**SUDEP)** include high seizure frequency, younger age, mental retardation and poly therapy.

Sleep disturbance is common in epilepsy. The nature of sleep disturbances in epilepsy is diverse, and the aetiologies are complex.

Patients with epilepsy commonly produce complaints of daytime sleepiness and poor quality of sleep. These problems are frequently attributed to antiepileptic drugs and seizures.

NORMAL HYPNOGRAM:

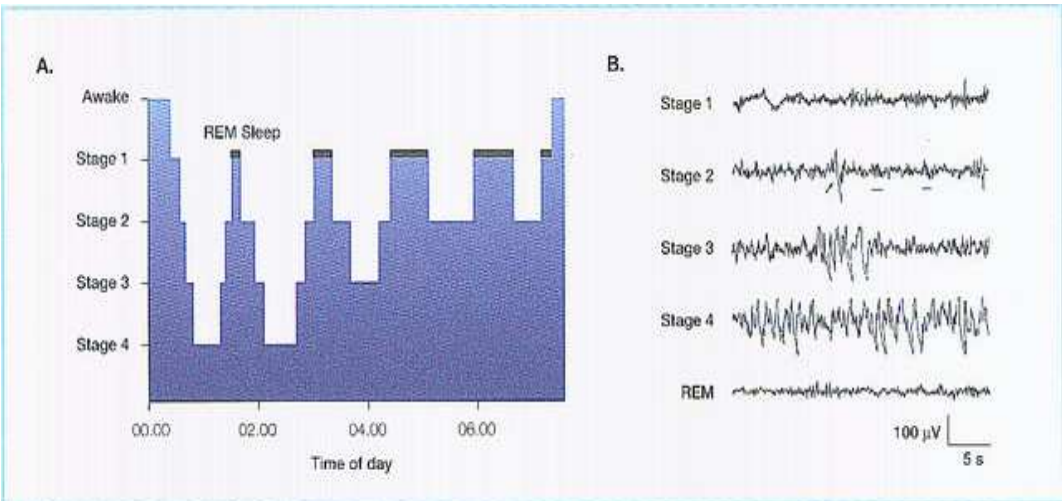
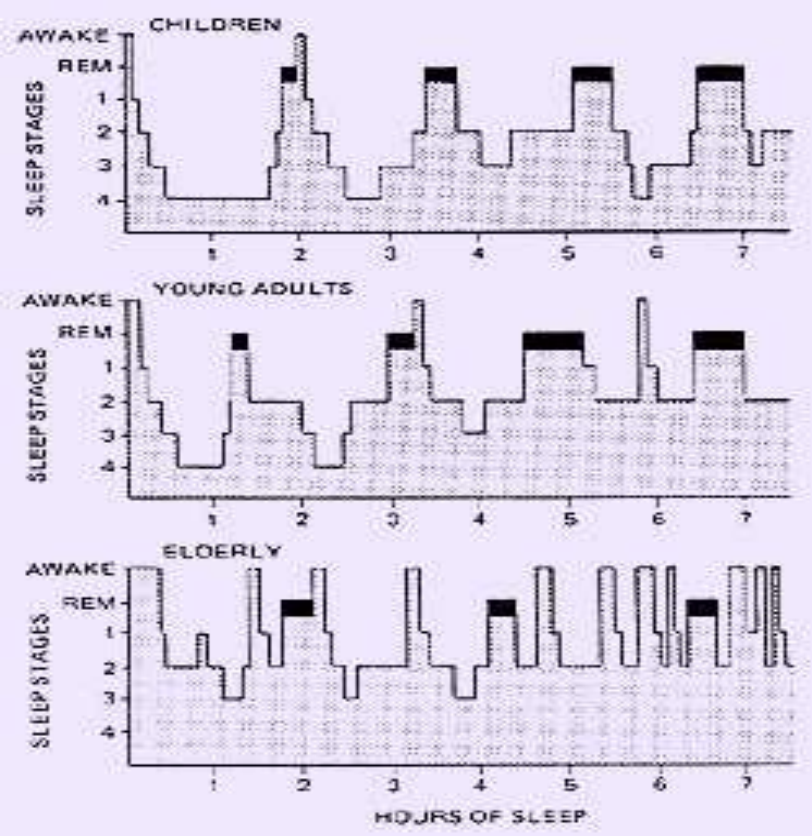


Figure 1. **Sleep stages: A. Hypnogram and B. Electroencephalogram.** Adapted with permission from Carskadon and Dement[2] and Kandel et al.[3]

During sleep, we mostly pass through five stages 1,2,3,4, and REM (Rapid eye movement) sleep. These stages progress in a sequence from stage 1 to REM sleep, then the cycle repeats from stage 1. Among these almost 50 percent of our total sleep time is

occupied by stage 2 sleep, about 20 percent by REM sleep ,and the remaining 30 percent in the other stages. Infants, on the other side, run through about half of their sleep time in REM sleep.

HYPNOGRAM ACCORDING TO THE AGE:



Hours of sleep against age

Children need more sleep per day in order to develop and function properly: up to 18 hours for newborn babies, with a declining rate as a child ages. REM sleep of a newborn is nearly 9 hours a day. After the age of five REM sleep component declines nearly more than two hours. 10 to 11 hours of sleep is required for school children as evidenced by studies1.

AGE AND CONDITION SLEEP NEEDS

Newborns (0–2 months)	-	12 to 18 hours
Infants (3–11 months)	-	14 to 15 hours
Toddlers (1–3 years)	-	12 to 14 hours
Preschoolers (3–5 years)	-	11 to 13 hours
School-age children (5–10 years)	-	10 to 11 hours
Adolescents (10–17 years)	-	8.5 to 9.25 hours
Adults, including elderly	-	7 to 9 hours.

1.1 PHYSIOLOGY OF SLEEP:

This is widely divided into two following Phases of Sleep according to the multiple electrophysiological recording parameters as Rapid Eye Movement (REM) and Non-Rapid Eye Movement (NREM).

1.2 STAGES OF SLEEP CYCLES:

The EEG pattern recorded during sleep varies in a cyclic fashion, which repeats in about every 90 minutes. There are about four cycles in normal 6 to 8 hours of sleep. In normal individuals, sleep cycle begins with slow-wave sleep or Non-REM sleep. There are four stages of slow wave sleep: stages 1 to 4.

A person when falls asleep, passes sequentially through these four Stages of increasingly deep sleep. After that, the sleep lightens and he enters into REM period. With completion of REM phase, sleep cycle completes. The REM phase is followed by the next new cycle, i.e. with stage 1 of non-REM sleep.

Thus, the cycle repeats in every 70 to 90 minutes. Throughout the night, people wake up briefly (called stage W) but are typically unaware of being awake. There are differences in the proportion of time spent in the various sleep stages in different age groups. Moreover, each individual has his or her own characteristic pattern. Usually, there is a predominance of deep slow wave sleep during the early part of the night, and the first REM sleep may occur after an hour.

REM stage becomes prevalent during the later part of the night. In general, REM sleep occupies about 25 per cent of total sleep period. The duration of REM sleep and stage 4 sleep decrease gradually with advancing age. Newborns and infants sleep about 18 hours a day of which 50% is spent in REM sleep.

1.3 EEG FEATURES OF SLEEP

In 1953, Aserinsky, Dement and Kleitman through EEG and polygraphic analysis described different phases of normal sleep characterized by EEG, autonomic and endocrine changes.

During wakefulness, EEG usually shows desynchronized, highfrequency, low amplitude known as beta waves in the range of 14 – 30 Hz. During quiet rest with eyes closed, waves range from 8 to 12 Hz, i.e. alpha waves.

1.4 ARCHITECTURE

Non-REM sleep:

Stage 1 : Alpha rhythm is replaced by high frequency low amplitude EEG waves.

Stage 2 : Appearance of sleep spindles and K complexes.

Stage 3 & 4 : Delta waves (slow wave)

(Commonly called as deep sleep)- Delta waves in EEG reflect synchronized oscillations of thalamocortical circuit activity.

REM sleep:

High frequency, low amplitude activity with PGO spikes. REM is characterized by rapid eye ball movement and profound atonia of other limb muscles (except for extraocular, inner ear and respiratory muscles).

Among these stage 2 sleep occupies50 percent of our total sleep time, REM sleep 20%, and the remaining 30 percent in the other stages.

Infants sleep on the other hand, occupies 50% of their sleep time in REM sleep. Recent evidences show that "theta oscillations during REM are driven by neurons in the proceruleus area in pons and atonia is caused neurons in the adjacent sublaterodorsal area. **(Lu et al, 2006).**These 'REM on' zones are inhibited by nearby 'REM off' area including ventrolateral PAG and lateral pontine tegmentum. 'REM on' area can also inhibit 'REM off' area. Mutual inhibitions between these areas produce 'flip-flop switch' that ensures sharp and complete transition between REM and NREM sleep".

REM switch is influenced by:

- Cholinergic neurons that promote REM sleep
- Noradrenergic and serotonergic neurons that inhibit REM sleep.

NREM Sleep-changes in EEG and consciousness (which is primarily a cerebral function) are classified into four stages respective to increasing depth of consciousness. Depth of unconsciousness will become more for as sleep progresses from

stage 1 to 4. EEG shows a progressively slower in frequency and higher-voltage pattern (furthermore called slow wave, delta wave)

NREM-Thinking and Body Activities:

Thinking in NREM is short, rudimentary and not able to recollect, Muscle tone is present, and Deep Tendon Reflex can be elicited. Chin and limb muscles exhibit EMG activities.

NREM sleep-autonomic changes:

Widespread decrease in autonomic activities, hypotension and decreased heart rate, and decreased generalized cellular metabolism are noted.

NREM sleep-Hormonal changes:

Growth Hormone, Cortisol and Prolactin secretion occurs mostly in NREM sleep. GH is secreted in 30 to 60 minutes after the beginning of sleep. Biochemical changes like increased serotonin activity is noted in NREM sleep.

Function of NREM sleep:

Main characteristics of NREM sleep are slow wave in EEG, decreased generalized metabolic activities and deep unconsciousness help revitalize the body. Hence, early night time period is occupied by NREM or slow wave sleep predominantly, while late night phase is occupied by REM which is lighter and dream filled.

EEG/EMG characteristics of REM sleep:

EEG is more active than NREM. Low voltage fast with ocular movement artifact is noted in EEG. EMG is relatively reflecting atonia in REM sleep related to flaccid muscles. While other body activities are as active as like that of awake state.

Latencies:

"Sleep latency is defined as the interval to fall asleep after retiring. Normal range is 10-20 minutes".

The occurrence of seizures can have profound effects on sleep architecture lasting longer than the post ictal period. Significant sleep interruption in epilepsy has been associated with impaired seizure control. All the aspects of sleep should be evaluated, and sleep disturbances should be treated as part of the total care of patients with epilepsy.

In epilepsy patients, formal sleep evaluation demonstrates significantly reduced REM and stage 4. Patients having nocturnal seizures show reduced sleep efficiency, increased time to first REM period.

Seizures can also influence changes in sleep. They demonstrates multiple sleep abnormalities, including increased sleep latency, fragmented sleep, increased awakenings and stage shifts, and an increase in stages 1 and

2 of non-rapid eye movement sleep (Foldvary -Schaefer and Grigg – Damberger 2009)[1]

Physiologic changes involved in the non rapid eye movement (NREM) sleep are associated with increased susceptibility to seizures.

Ghrelin is a 28 aminoacid peptide hormone that is secreted mainly by stomach cells with small amounts secreted by other cells (hypothalamus), that is a growth hormone secretagogue, and that has been implicated in the, stimulation of fat storage and food intake.

Ghrelin is a hormone with multiple functions(korbonits et al 2004)[71]. It has been reported to affect cardiac and gastrointestinal function, carbohydrate metabolism, adipose and reproductive tissue, cell proliferation and behaviour as well as affecting sleep and pituitary hormone axis function.

Ghrelin promotes slow-wave sleep in humans (**Weikel, J.C et al)** [2] Short sleep duration is associated with high levels of ghrelin and obesity. An inverse relationship between the hours of sleep and blood plasma concentrations of ghrelin exists: as the hours of sleep increase, ghrelin levels lower and obesity is less likely.

REVIEW OF LITERATURE

HISTORY OF EPILEPSY:

Epilepsy is an enigmatic disorder which had different outlook and approach in different era.

The term Epilepsy originated from the Greek word “seize” or attack and probably was derived from the idea of the individual being seized or attacked by Gods (**Temkin O et al 1971**)[3].

In fifth –century B.C, Hippocrates, the father of medicine made several observations regarding Epilepsy, which was then referred to as the “Sacred disease (**Eadie MJ et al 2001**)[4]. He attributed the cause of Epilepsy to a physical condition, specifically a hereditary excess of phlegm in the brain. At that time epilepsy was attributed to a variety of causes, including smiting by the Gods, demonic possession, prophetic trances, insanity etc.

Another Greek physician, Galen used the term “aura” to describe the feeling ‘like a cold breeze’ that travelled up the body from the log prior to a seizure.

In the Christian era in Europe ,epilepsy was no longer considered the “Sacred disease”, but thought to be due to evil possession .In the seventeenth century ,two famous anatomists,

Franciscus sylvius and Thomas Willis carefully studied the brain and developed theories to explain the epileptic phenomenon.

However, the outlook changed with the dawn of scientific medicine in the 19th century when Sir Charles Locock in England, observed that the administration of bromides seemed to cure epilepsy in young adult women. Following this phenobarbitone and phenytoin evolved as highly effective anticonvulsants. In the 1940 s and 1950 s, there was a flurry of drug discoveries. Since 1990, a variety of pharmacologic, non pharmacologic and surgical approaches to treatment have been introduced.

With improved treatment available for epilepsy, diagnosis became an increasingly important issue and the recording of abnormal brain electrical activity became mandatory. In 1924, Hans Berger from university of Jena psychiatric clinic of Otto **(Berger H, et al 1969)** [5] made the breakthrough of first scalp recording of normal brain electrical activity and called the recording an “Elektenkephalogram”.

From 1950 s, neuroscience research involving epilepsy quickened, leading to remarkable discoveries about genetics, pathology, physiology, diagnostic tools and treatment of epilepsy.

EPIDEMIOLOGY:

Next to stroke and Alzheimer's disease, Epilepsy is the third most common neurologic disorder.

Approximately 2 million (0.5-1.5% of population) peoples suffered from this disorder in the United States. Each year 50 per 1, 00,000 persons in the United States will be diagnosed with epilepsy, frequency of seizures more in of new cases occurring among the children<5 years and adults>65 years of age.

Epilepsy is one of the most common and serious neurological disorders affecting 65 million people globally (**Thumaran et al)** [6]. It affects 1% of the population by age 20 and 3% of the population by age 75(**Holmes et al**) .Most of those with disease nearly 80% are in the developing world **(Newton CR et al)** . It is more common in males than females.

5-10 per 1000 peoples are having active epilepsy now. It means patient had at least one seizure in the last 5 years. Poverty is one of the important risk factor for epilepsy. In the developed world, either it starts in the young or in the old. But in the developing world its onset is more common in older children or young adults due to higher rates of trauma and infectious disease[i].

Epilepsy begins each year in 80-140 per 100,000 persons in developing countries and in developed countries 40-70 per 10,000 persons (**Sander JW et al 2003).**

Approximately 181,000 new cases of epilepsy and seizures occur each year. About 2.3 million Americans have epilepsy. Half of the people with epilepsy develop seizures by the age of 25; however anyone can get epilepsy at anytime.

Between 1.5% and 5% of Americans have seizure at some occasion in their lives and about 0.5% have epilepsy, according to the National Institutes of Health.

Aetiology of Seizures and Epilepsy:

Seizures occur as result of a shift in the normal balance of inhibition and excitation within the central nervous system (CNS). The normal human brain has a tendency to develop seizures under certain circumstances, and there are differences between individuals in the susceptibility for seizures. There are various endogenous factors involved in the seizure occurrence. Normal brain development also plays important role, since the brain appears to have different seizure thresholds at various maturational stages. There are certain conditions that have a high possibility of

developing a chronic seizure disorder. Seizures are episodic. Patients with epilepsy have seizures intermittently and, depending on the underlying reason, many of them are completely normal for few months to years between seizures. Certain precipitating factors are responsible for causing the single seizure in some persons without epilepsy. This implies a dynamic interplay between endogenous factors, epileptogenic factors, and precipitating factors.

CAUSES OF SEIZURES ACCORDING TO AGE:

Neonates (<1 month):

- Perinatal hypoxia and Ischemia
- Acute CNS infection
- Intracranial haemorrhage and trauma
- Metabolic impairments (hypo glycaemia, hypocalcaemia, hypo magnesemia, vitamin B6 deficiency)
- Genetic causes

Infants and children (>1 month and < 12 years):

- Febrile seizures Genetic disorders (metabolic, degenerative, primary epilepsy syndromes)
- CNS infection
- Developmental disorders
- Idiopathic

Adolescents (12-18 years):

- Trauma
- Infection
- Brain tumour
- Genetic disorders
- Idiopathic

Young adults (18-35 years):

- Trauma
- Alcohol withdrawal
- Illicit drug use
- Idiopathic

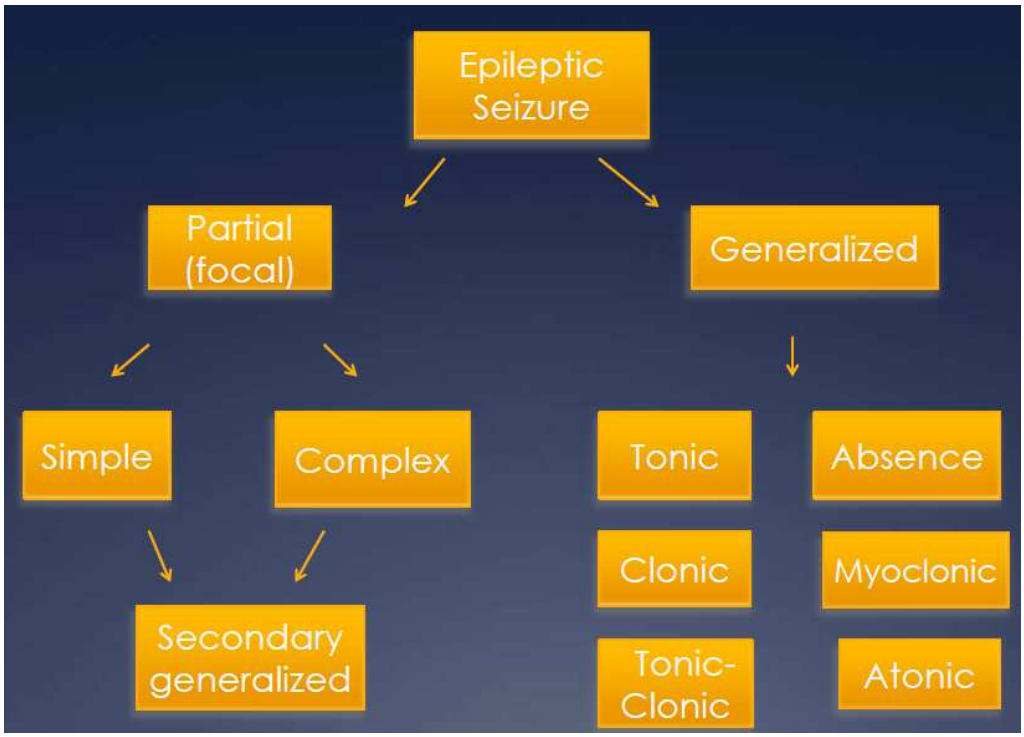
Older adults (>35 years):

- Cerebrovascular disease
- Brain tumour
- Metabolic disorders (uraemia, hepatic failure, electrolyte abnormalities, hypoglycaemia, and hyperglycaemia)
- Alcohol withdrawal
- Alzheimer's disease and other degenerative CNS diseases
- Idiopathic

The most common variety of seizures arising in late infancy and early childhood are **febrile seizures**, which are seizures associated with fever but not associated with any evidence of CNS infection. The overall prevalence is 3-5% and even high in some parts of world. Patients often have a history of epilepsy in their family. Febrile seizures usually occur between 3 months and 5 years of age and peak between 18 and 24 months. The seizures usually occur during the rising phase of fever that is the first day of fever rather than the further days.

Febrile seizures divided into two types. First type is simple febrile seizure is a single, isolated event, symmetric and brief in its course. Second type is complex febrile seizures manifests as repeated seizure activity, lasts more than 15 minutes. Approximately < 10% peoples have three or more episodes and one third of patients will have a recurrence. Recurrences are more if the febrile seizures occur in the first year itself. Complex febrile seizures have a tendency to develop into epilepsy approximately 2-5% of peoples.

TYPES OF EPILEPTIC SEIZURES:



International classification of epileptic seizures:

This classification is proposed by International League against Epilepsy (ILAE) and based on the clinical expression and electroencephalographic features of seizures during and between the episodes, approved in September 1981. It is mainly divided into partial and generalized seizures.

Partial seizures (seizures beginning locally):

a. **Simple partial seizures** (consciousness not impaired)

1. With motor symptoms
2. With somato sensory or special sensory symptoms
3. With autonomic symptoms
4. With psychic symptoms

b. **Complex partial seizures** (with impairment of consciousness)

1. Beginning as simple partial seizures and progressing to impairment of consciousness.

a) With no other features

b) With features as in A1-4

c) With automatisms

With impairment of consciousness at onset

a) with no other features

b) with features as in A1-4

c) with automatisms

C. Partial seizures secondarily generalized

II. GENERALIZED SEIZURES (bilaterally symmetrical and without local onset)

A. 1. Absence seizures
2. Atypical absence seizures

B. Myoclonic seizures

C. Clonic seizures

D. Tonic seizures

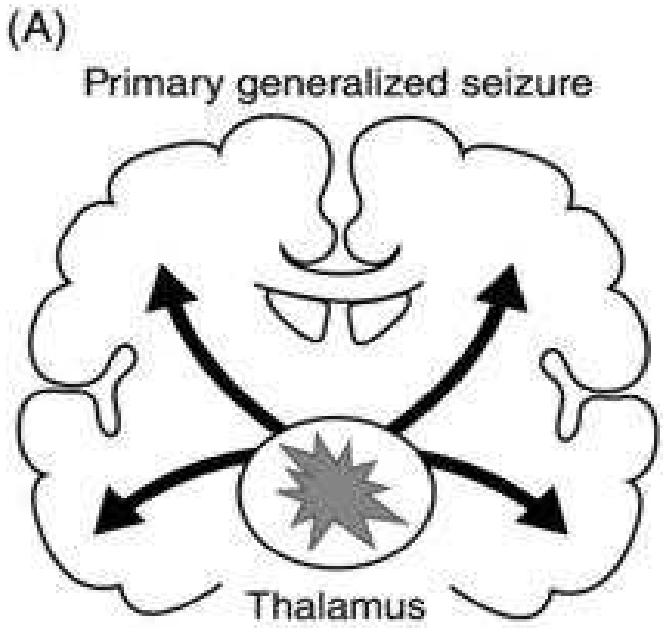
E. Tonic – clonic seizures

F. Atonic seizures

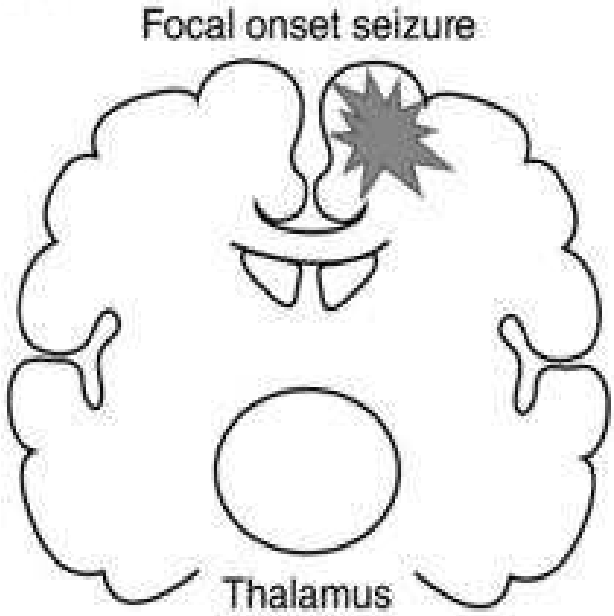
III UNCLASSIFIED EPILEPTIC SEIZURES

In partial seizures the abnormal electrical impulses start in a localized area of the brain. Based on the part involved in the brain, clinical manifestations are expressed. These impulses may remain localized or they may spread to other areas of brain and then the seizure becomes generalized called **secondarily generalized seizures**.

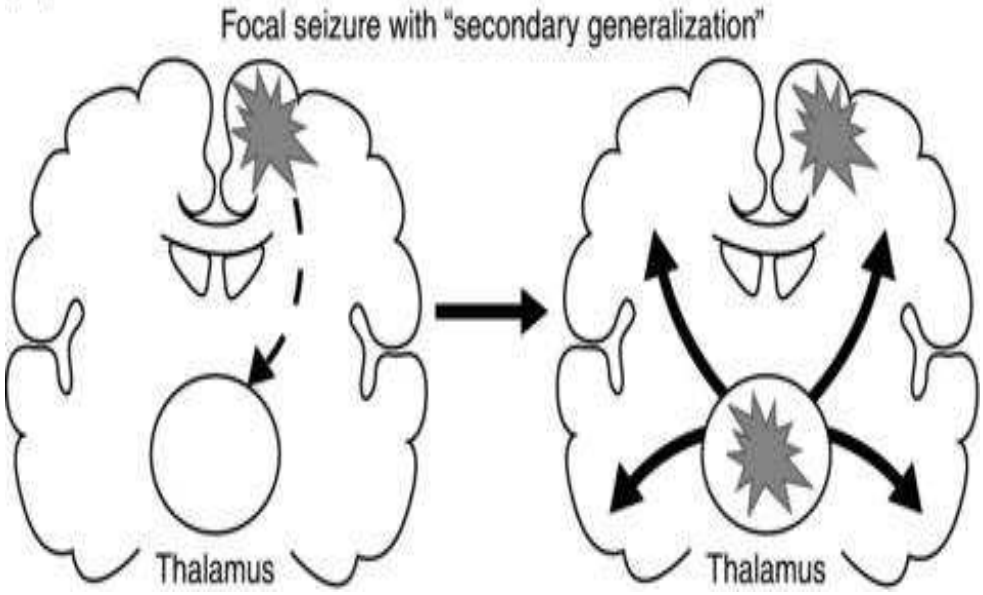
On the other hand, **generalized seizures** are starts from both the hemispheres simultaneously, generalized in onset.



(B)

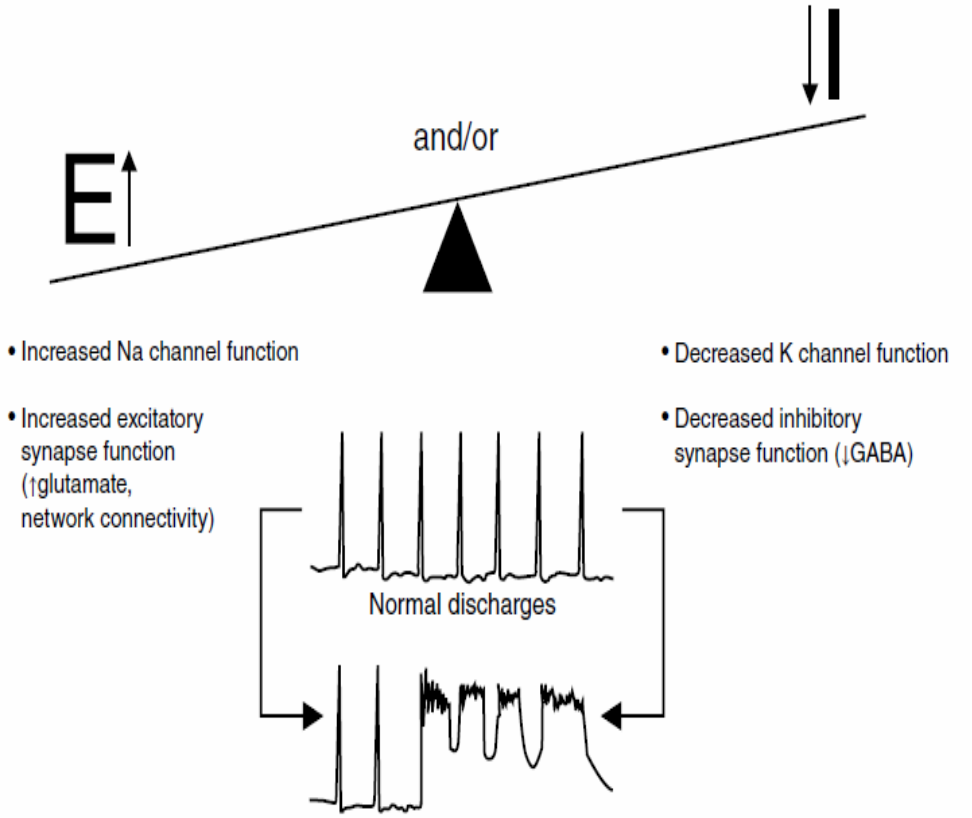

Focal onset seizure
Thalamus

(C)


Focal seizure with "secondary generalization"
Thalamus
Thalamus

During lifetime, one patient may have a combination of seizure types, not necessarily have only one type of seizure. Depending on the age and maturation of the brain, the type may change. A definite aura is an indication of seizure of focal onset.

pathophysiological processes leading to epilepsy:



Epileptic discharges

This picture shows that seizure generation results from increased excitation and decreased inhibition, or both.

Mechanisms of Seizure Initiation and Propagation (Harrisons text book of internal medicine) 7:

There are two phases in the seizure occurrence. First seizure initiation phase characterized by two concurrent events in an aggregate of neurons.

1. High- frequency bursts of action potentials
2. Hyper synchronization

The bursting activity is due to long-lasting depolarization of the neuronal membrane due to influx of extracellular calcium (Ca2+), which leads to the opening of sodium channels (Na+), influx of Na+, and generation of repetitive action potentials. This is followed by a hyperpolarizing after potential mediated by gamma amino butyric (GABA) receptors or potassium channels, depending on the cell type. The synchronized bursts from a number of neurons result in a so-called spike discharge on the EEG.

This spread of activity normally prevented by intact hyperpolarization and inhibition produced by surrounding inhibitory neurons. With adequate activation there is a recruitment of surrounding neurons via a number of synaptic and non synaptic mechanisms, including:

1. Excessive extracellular k+, which blunts hyper polarization and depolarizes neighbouring neurons.
2. Calcium ions accumulated in the pre synaptic terminals, leading to increased neurotransmitter release
3. Depolarization-induced activation of the (NMDA receptors) N-methyl-D- aspartate subtype of the excitatory amino acid receptor, which causes additional Ca2+ influx and neuronal activation
4. Ephaptic interactions related to changes in tissue osmolarity and cell swelling.

The recruitment of a sufficient number of neurons leads to the propagation of seizure activity into contiguous areas through cortical connections and to more distant areas via long commissural pathways. There are many mechanisms for altering a neurons propensity to have bursting activity and many factors control the neuronal excitability.

There are two types of mechanisms.

First one is the intrinsic mechanisms; include changes in the conductance of ion channels, cytoplasmic buffering, second-messanger systems, and protein expression as determined by gene transcription, translation, and posttranslational modification.

Second extrinsic mechanisms are changes in the amount or type of neurotransmitters present at the synaptic level, modulation of receptors by extracellular ions, and temporal and spatial properties of synaptic and non synaptic input. Astrocytes and oligodendrocytes, have an important role in many of these mechanisms.

Mechanisms of Epileptogenesis:

Epileptogenesis refers to the transformation of a normal neuronal network in to one that is chronically hyper excitable. There is often a delay of months to years between an initial injury and the first seizure. The injury appears to initiate a process that gradually decreases the seizure threshold in the affected region until a spontaneous seizure occurs. In many idiopathic and genetic forms of epilepsy, epileptogenesis is determined by developmentally regulated events.

GENETIC CAUSES OF EPILEPSY:

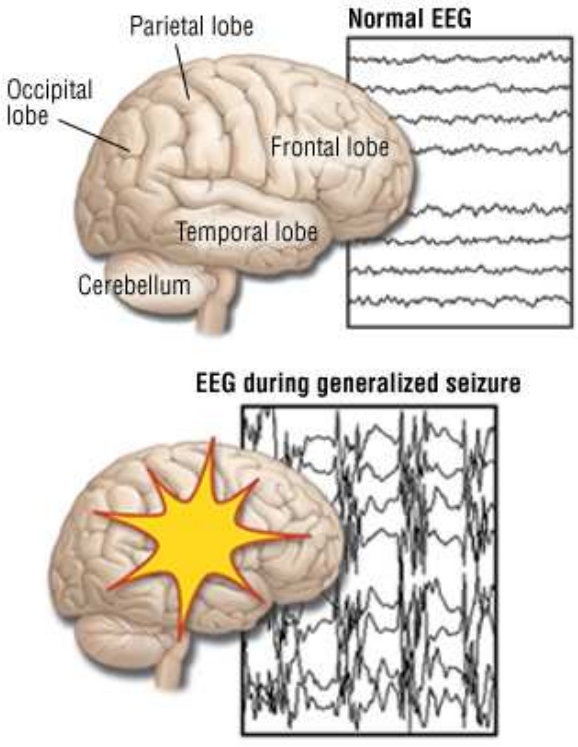
Identification of Genetic mutations associated with epilepsy syndromes are the most important recent research progress in epilepsy. Most of the inherited, idiopathic epilepsies due to mutations affecting ion channel function.

Identification of multiple susceptibility genes that underlie the common forms of epilepsy is the current challenge of epilepsy research. Recent studies suggest that chromosomal micro deletions and ion channel mutations may be the cause of epilepsy.

TONIC- CLONIC SEIZURES:

These seizures are also called grandmal seizures. It is the most typical type seen in adults. More than half of the patients with epilepsy may experience a generalized tonic – clonic seizures in the course of the disease process.

EEG FEATURE OF GTCS:



Prior to the attack, some patients may experience vague symptoms called an ‘aura’. This may be a taste or a smell, or just ‘feeling strange’. It may last for a few seconds, which may be enough for the patient to be able to lie down, thus preventing injury due to falling during seizure attack. However most of the patients do not have any warning symptoms.

The typical seizure of this type has three phases.

The **first phase** is the tonic phase, which last for about 10 to 40 seconds. The patient become vey rigid, as all the muscles in the body undergo tonic sustained contractions. The patient falls rigidly and as the laryngeal and respiratory muscles are also contracted, may let out a cry or grunt as air is forced out of the chest through the vocal chords. During this time the patient becomes cyanotic due to insufficient respiration.

This is followed by the **second phase** called clonic phase, during which the muscles go in to strong random contractions. This limb jerking may be accompanied by faecal and urinary incontinence, and there may be tongue and biting and frothing at the mouth. Breathing is inefficient and jerky and there is tachycardia. This phase last for 2 to 3 minutes, although it may extends longer.

The **third phase** is a coma, in which the patient breathing becomes irregular. Their colour becomes normal. The length of this phase is related to the duration of previous tonic clonic phases, while recovery the patient may appears to be confused and have a headache. During that seizure attack there will be reduction of respiration leads to poor oxygenation of the blood. There is an accumulation of lactic acid in brain. The probable cause of the coma is hypoxia and acidosis.

Blood investigation shows increased pH, decreased $pO_{2,}$ increased creatinine phosphokinase and serum prolactin levels.

Repeated seizure attacks can lead to neuronal degeneration which is due to the excessive synthesis of glutamate. This can cause cell death due to excitotoxicity.

SUDEP: (sudden unexpected death in Epilepsy)

Sudep is the non accidental death in patients with epilepsy. on autopsy, cause of death is unknown.

The rate of SUDEP is approximately 1 death in 1000 people with epilepsy per year. It is the leading cause of death in people with uncontrolled epilepsy. Its cause is multifactorial. The main risk factor for sudep is frequent seizures, especially in grandma

seizures (generalized tonic-clonic seizures). Other risk factors are long duration and early onset of epilepsy, fails to take antiepileptic medications, young age (20-40), IQ< 70. Some studies explain higher risk of SUDEP seen in patients taking multidrug therapy.

GHRELIN HORMONE:

Introduction:

(Ghrelin-Growth **H**ormone **Rel**ease **In**ducing)

The name Ghrelin is derived from Growth Hormone Release Inducing hormone. This hormone has its role as a growth hormone releasing peptide.

Discovery of Ghrelin hormone:

It was discovered after the discovery of its receptor (Ghrelin receptor-Growth hormone secretagogue receptor (GHSR) by **Howard et al**[8]. It was discovered in 1996, reported later in 1999 and its natural ligand was not known. Three years later (1999), its natural ligand, **Ghrelin** identified by **Kojima et al**[9] from the rat stomach.

Ghrelin is the most potent circulating orexigen, and its plasma levels are elevated prior to the meals and it stimulates

feeding (**Wren et al 2001**)[85]. So, **ghrelin is also called hunger hormone** .Ghrelin is encoded by the GHRL gene.

Figure Aminoacid Sequence of Ghrelin

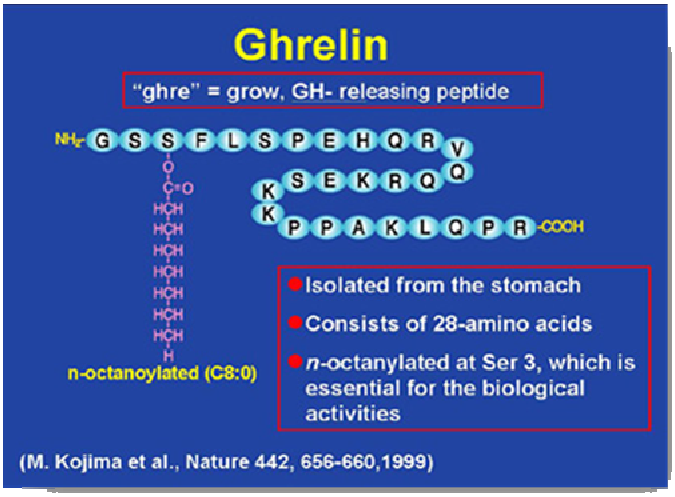
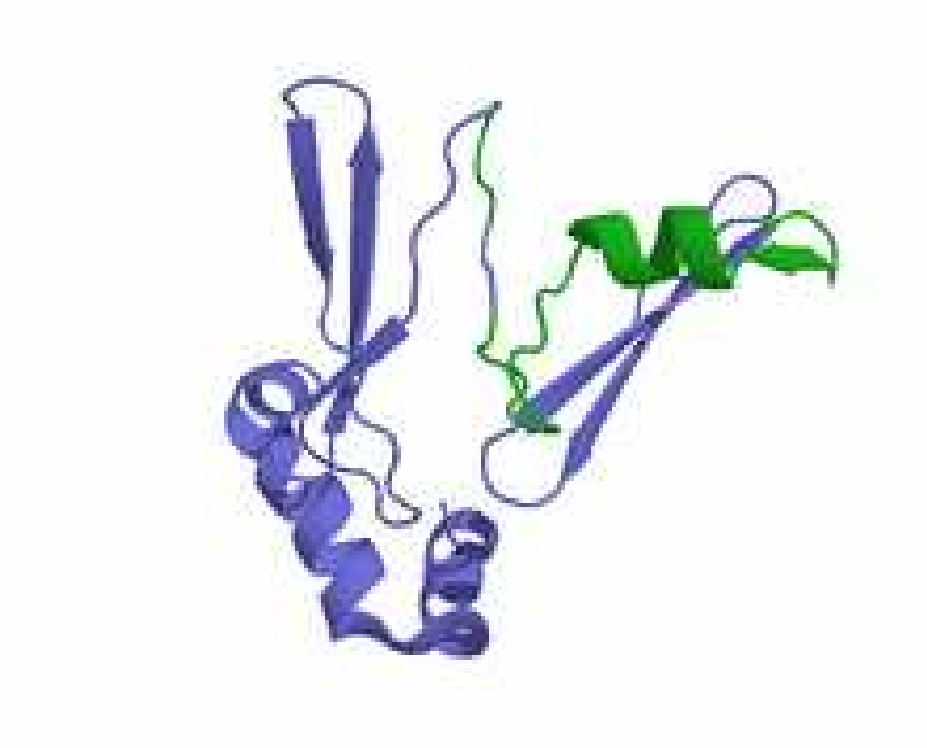


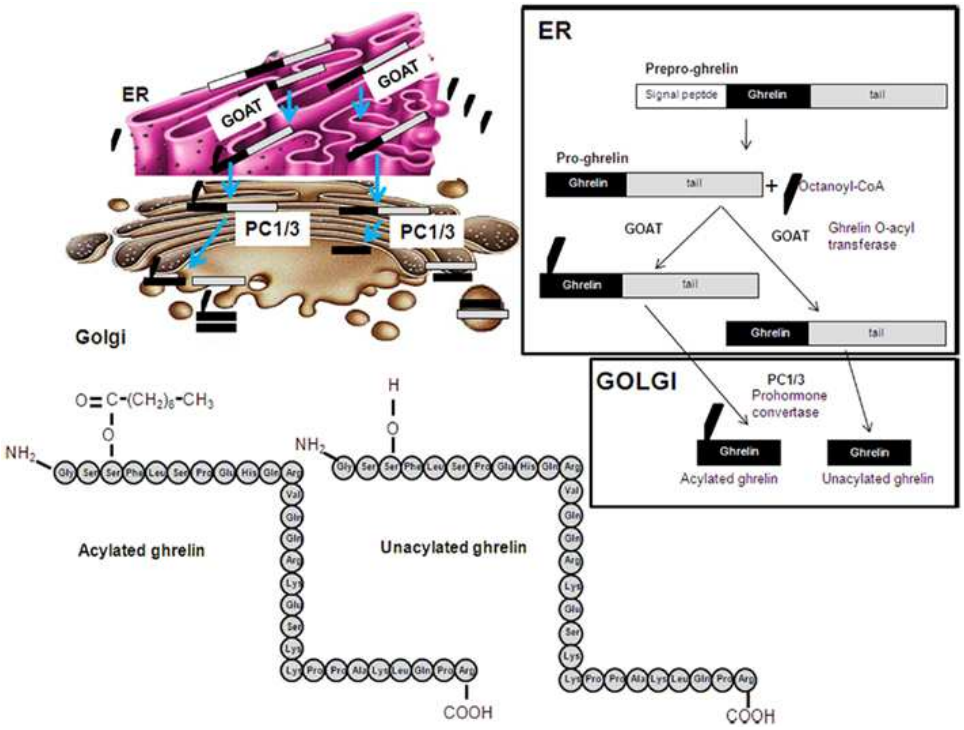
Figure GHRELIN AND PREPROGHRELIN



Green and blue-Pre pro ghrelin, Green-Ghrelin

Figure ***Formation of Ghrelin from prepro-Ghrelin.***

Types of Ghrelin acylated and unacylated Ghrelin.



The ghrelin gene contains preproghrelin - coding exons (exons1-4). During processing, a 23 amino acid secretion- signal peptide is cleaved from the 117 amino acid preprohormone (N-terminus), resulting in 94 amino acid proghrelin . This peptide further cleaved and gives rise to 28 amino acid ghrelin peptide and a 66 amino acid C-terminal propeptide, C-ghrelin (**Pemberton et al 2003).** Following the cleavage from proghrelin, the ghrelin peptide can be posttranslationally octanoylated at its third residue serine, by enzyme **ghrelin O-acyl transferase(GOAT)**(**Gutierrez et al**

2008)[10]. This modified form is referred to as ghrelin. A non-octanoylated form of ghrelin (unacylated ghrelin, des-acyl ghrelin, des-ghrelin) circulates in the higher level in the blood (**Holmes et al., 2009)[84].** It does not bind with its receptor (GHSR 1a), as previously considered as biologically inactive.

GOAT:

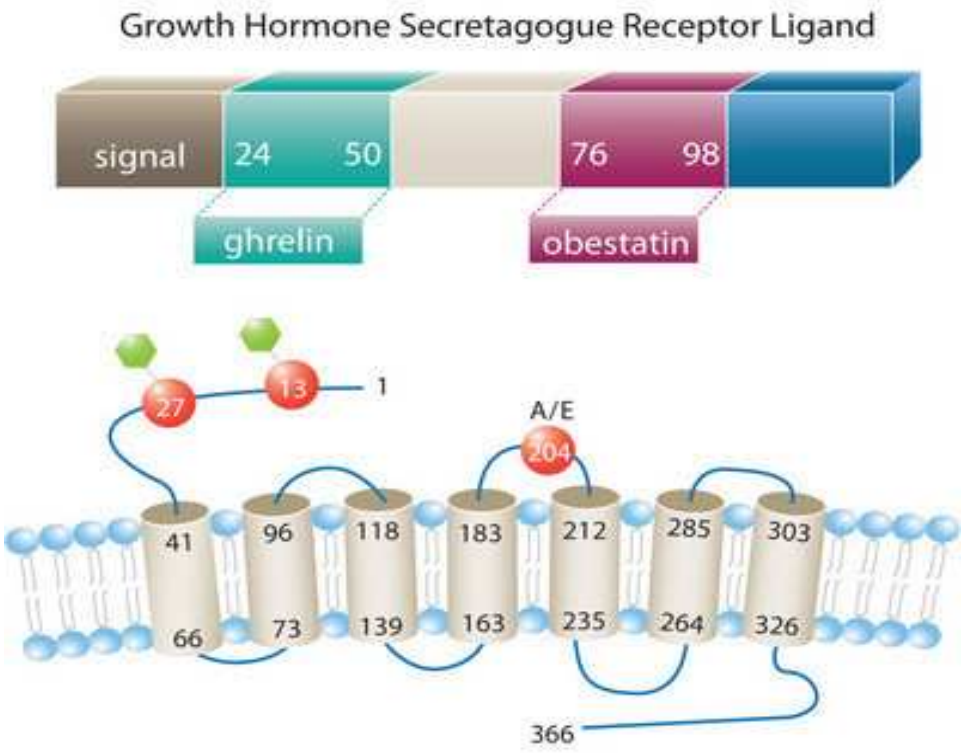
GOAT, enzyme responsible for octanoylation of ghrelin is a member from the membrane- bound O-acyl transferase (MBOAT) family of enzymes, encoded by the MBOAT4 gene. It is a hydrophobic, membrane-bound enzyme with 8 domains, localised to the endoplasmic reticulum (**Yang et al 2008a)[11]**. It is co expressed with ghrelin in the cells of stomach and in the pancreatic islets and other sites including chondrocytes (**Gomez et al 2009)[88].** This enzyme is an attractive and specific target for the modification of octanoylation (**Yang et al., 2008b)[11],** also it could provide a target for development of drugs to prevent weight gain, obesity, insulin resistance **(Barnett et al 2010)[89].** It also plays an important role in the regulation of metabolism and lipid - sensing, in the gut and links between energy intake with endocrine balance (**Kirchner et al 2009)[87].**

This c-ghrelin (has 66 amino acids) cleaved to give **obestatin**

Relation between ghrelin and obestatin:

Obestatin , which is C- terminally amidated and also it has opposite effects to ghrelin hormone on food intake(**Zhang et al., 2005**). It has multiple roles including, adipogenesis, in sleep, pancreatic homeostasis and cancer (**Seim et al[15].,).**

Figure Formation of Obestatin from Ghrelin



When octanoic acid (caprylic acid) is linked to serine posttranslationally at the third position by the enzyme ghrelin O-acyltransferase (GOAT) ,brings ghrelin becomes active. This enzyme located on the cell membrane of ghrelin cells in the pancreas and stomach[ii].

Ghrelin is a peptide hormone produced by ghrelin cells in the gastrointestinal tract which acts as a neuropeptide in the central nervous system. Ghrelin cells are found in oxyntic glands (20%), pyloric glands, small intestine. They also produce another food intake limiting hormone **Nesfatin-1(**Inhoff T et al[12]).They also present in lungs, pancreatic islets, gonads, adrenal cortex, placenta, kidney. They are ovoid cells with granules (**Grube D et al,)[13].** They have gastrin receptors.

Other names of ghrelin cells are A-like cell, X cell, X/A- like cell(rat), Epsilon cell(pancreas), P/D sub 1cell(humans**)(Zigman JM et al)[14].**

Growth Hormone Secretagogue Receptor:

GHSR 1a is a classical, 7- transmembrane domain, G protein-coupled receptor.

The ghrelin hormone secretogogue receptor (GHSR1a) is involved in so many functions of ghrelin. It includes stimulation of growth hormone release, regulation of motility and secretion of gastro intestinal system, increase in hunger, regulation of immune function and also has roles in sleep and memory. These receptors are situated in high density in the hypothalamus, pituitary, throughout the gastrointestinal tract and on the vagus nerve (**Seim I et al)[15].**

Another form is GHSR 1b isoform is thought to be inactive because it does not bind ghrelin and ghrelin does not activate signalling via this receptor (**Feighner et al., 1998**)[86]. It is over expressed in lung cancer. Although its function is not clear, it act as a negative regulator of GHSR 1a by reducing its cell surface expression and constitutive signalling **(Leung et al., 2007)[17].** Its over expression in many cancers indicates, its clinical significance (**Barzon et al., 2005)[90].**

The non-octanoylated form is DESACYL GHRELIN**.** This form does not activate the GHSR receptor, but it has involved in other actions such as cardiac function, appetite stimulation, anti ghrelin, inhibition of hepatic glucose output.

GHRELIN AND LEPTIN:

Ghrelin hormone discovered 7 years after **Leptin** hormone. Leptin is also a hormone involving in appetite regulation. It has opposite effect in appetite regulation in relation to ghrelin[68].

Figure Relation between Ghrelin and Leptin

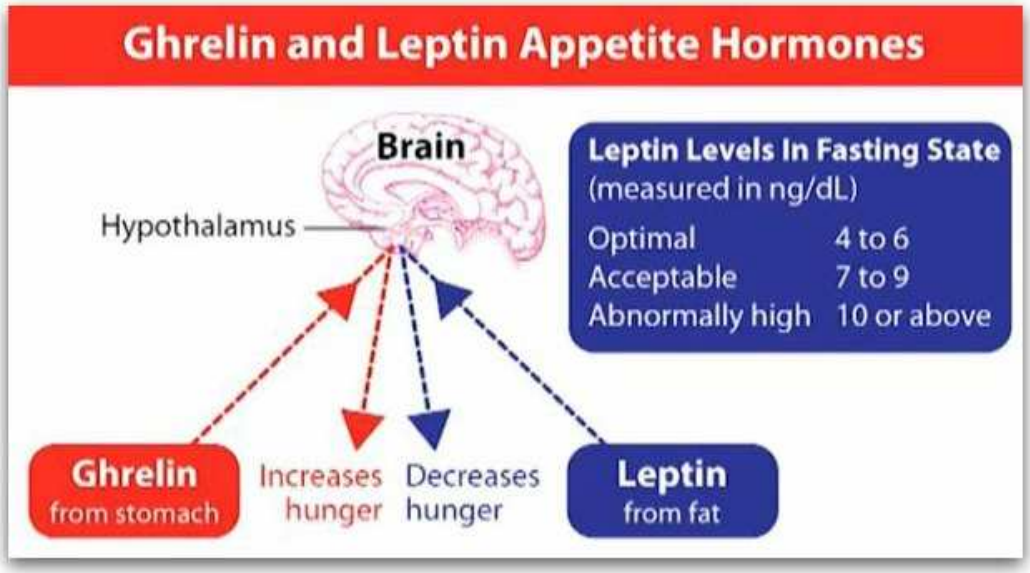
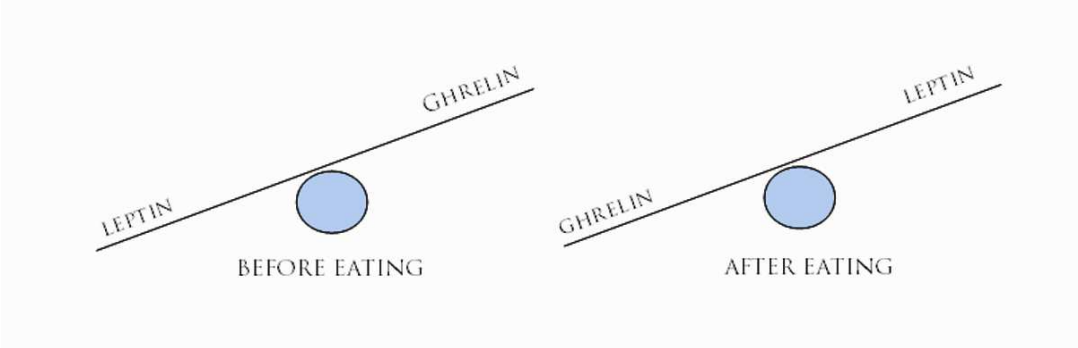
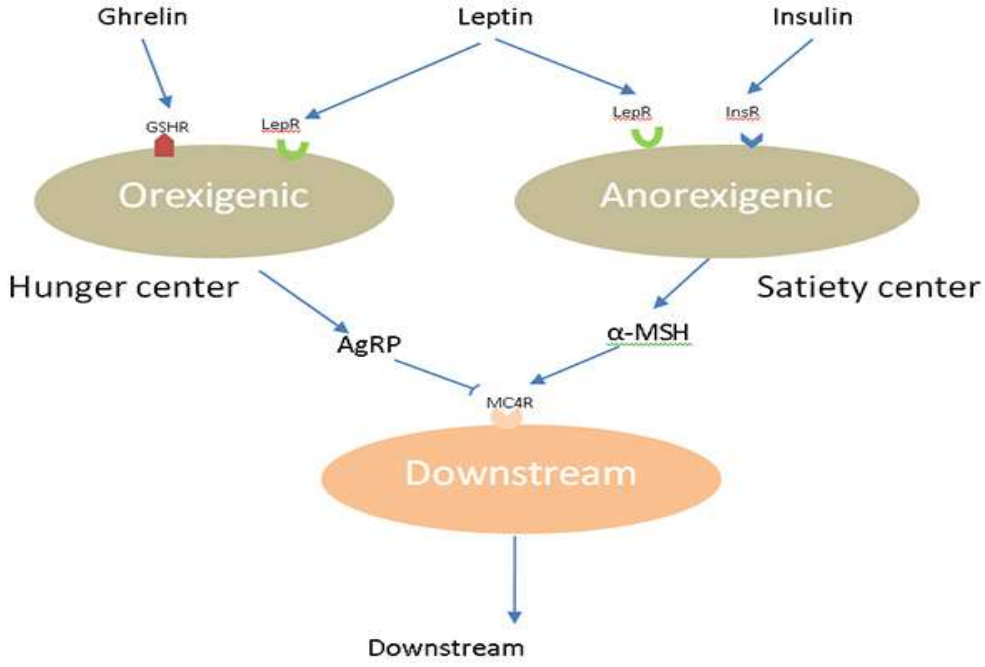


Figure Ghrelin and Leptin levels in relation to meals:



Orexigenic action of ghrelin:



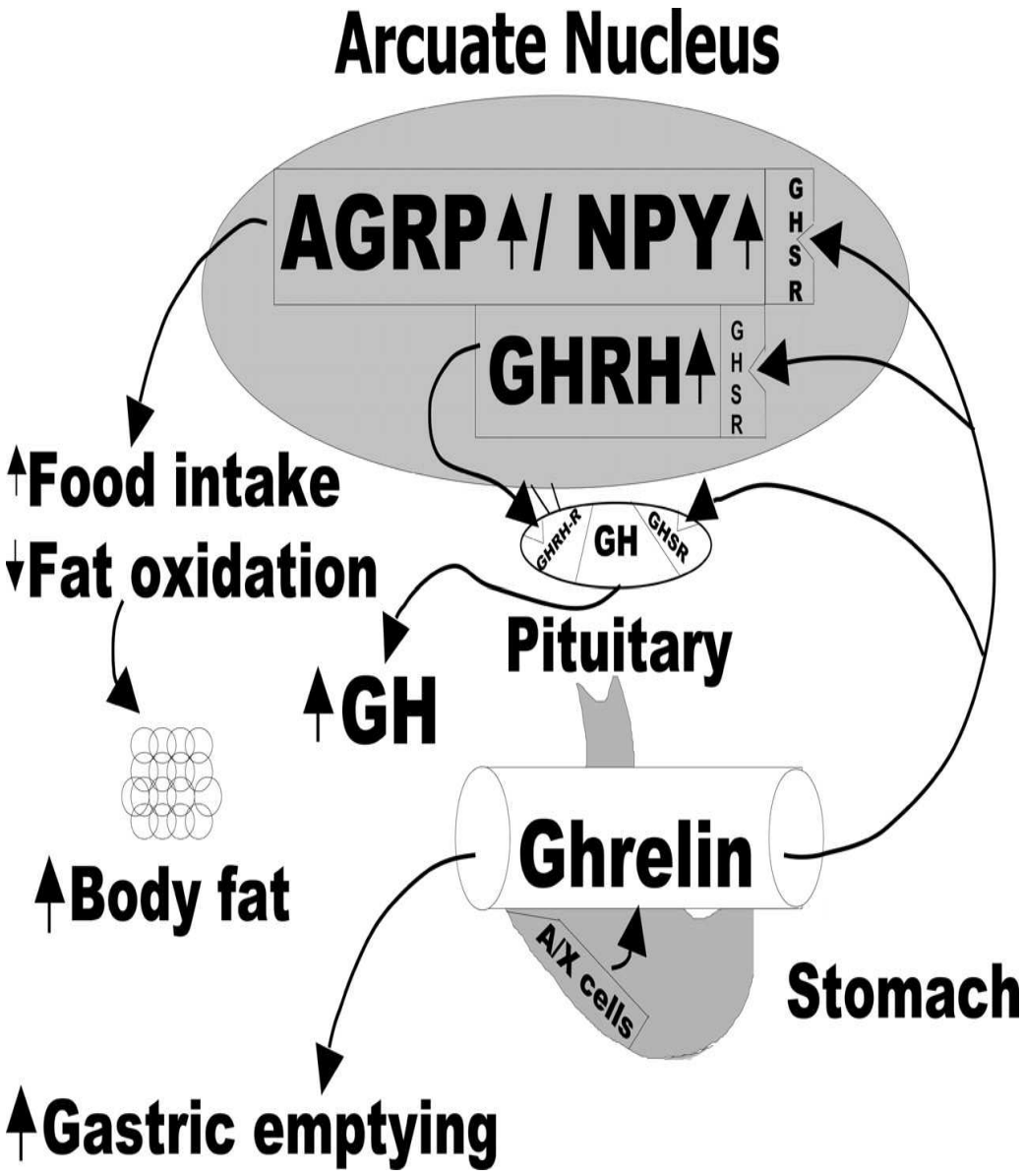
ACTIONS OF GHRELIN HORMONE :

Role in the nervous system;

1.Sleep:

There is an inverse relationship between the plasma concentration of ghrelin and hours of sleep. Short sleep duration is associated with increased ghrelin levels and obesity. Its levels become lower when the hours of sleep increases and the possibility of obesity also reduced[59].

Figure Functions of Ghrelin



2. Learning and memory:

In the brain hippocampus plays a significant role in the learning and the cognitive adaptation to changing environments. From the blood stream, ghrelin may enter the hippocampus, altering the nerve cell connections and also altering memory and learning proved in animal models. It is suggested that learning may be best when the stomach is empty during day time, because ghrelin concentrations are at high level (Diano et al,2006)[54] , (Ateha et al,2009)[55].

3. Role in prevention of depression:

Its role in depression is demonstrated by animal studies.(Spencer et al,2012)[57.]

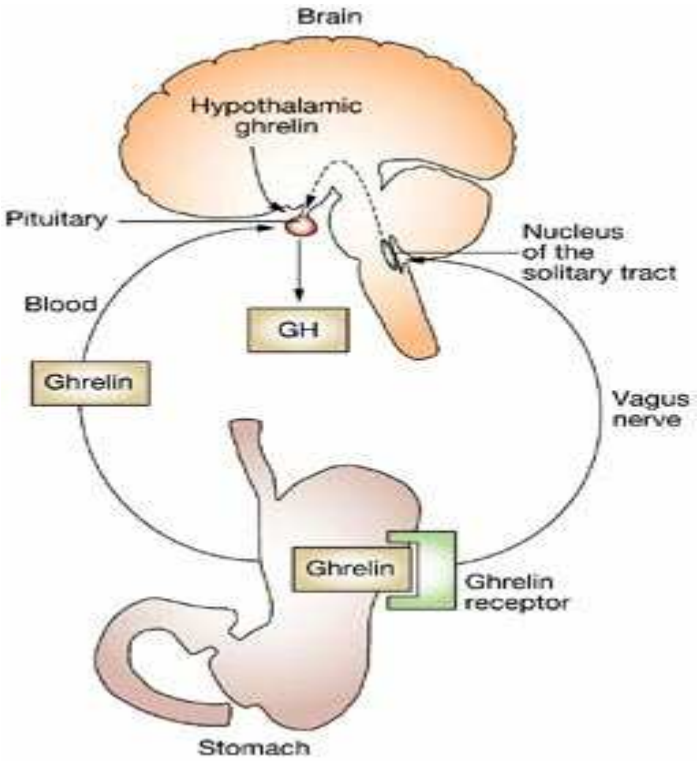
4. Role in stress:

Serum ghrelin levels are raised in stress even in the absence of adrenal hormones. This is necessary for learning of fear during stress situations. (Meyer et al, 2013)[60]

5. Role in growth hormone release:

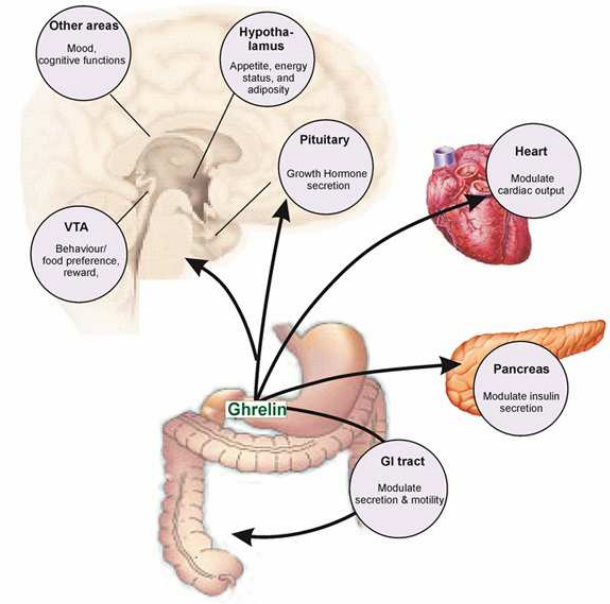
It has important role in growth hormone release.

ROLE OF GHRELIN IN GROWTH HORMONE RELEASE:



Gastro intestinal tract:

It promotes the proliferation of intestinal cells and inhibits apoptosis during oxidative stress (Wasseem et al 2004)[45.46].



It enhances anti inflammatory mechanisms, thus involved in the treatment of inflammatory conditions in gastro intestinal system. Also it has regenerative tendency, useful in treatment of mucosal injuries in the stomach(Ishres et al)[49].

On pancreas:

Ghrelin acts via its receptor in the pancreas to inhibit glucose induced insulin secretion.

In glucose metabolism, it has a gluco regulatory action(Heppner et al 2014)[53]

Role in obesity:

Its level low in obese persons except in prader-willi syndrome associated obesity. (Cumming DE et al 2002)[65]**Lack of sleep enhances ghrelin and decreases leptin level leading to obesity due to excessive production of hunger.**

In anorexia:

Ghrelin levels are high in anorexia nervosa and cancer induced cachexia patients.(Misra M et al 2014)[68]

Role in immune system:

Ghrelin gene products have actions on auto immunity**.(Prodam et al)[64]**

Role in reproductive system:

It has inhibitory action on gonadotropin-releasing hormone secretion and causes decreased fertility. (Comninos AN et al 2014)[62]

Role in fetus and neonates:

Ghrelin hormone synthesized by the fetal lung and promotes lung growth.(Santos M et al)[63] One study shows a correlation between ghrelin levels and birth weight by using cord blood levels.(Yokota I et al 2005)[43]

THERAPEUTIC APPLICATIONS OF GHRELIN:

1. Growth hormone deficiency
2. Eating disorders
3. Anorexia nervosa, Bulimia nervosa, Prader-willi syndrome
4. Gastro intestinal disease (Wer et al 2008)[47] (Gonzalez ray et al)[48]
5. Cardio vascular disease: Heart failure, dilated cardiomyopathy
6. Osteoporosis
7. Aging
8. Chronic wasting syndrome - Cachexia, AIDS, post operative patients.

ROLE OF GHRELIN IN EPILEPSY:

Ghrelin levels after epileptic seizures, results were controversial both in human and animals.

Interplay between epilepsy and the endocrine system exist. And Epilepsy and antiepileptic drugs affect hormonal system and neuroendocrinal system.

RELATION BETWEEN SLEEP AND EPILEPSY:

Inadequate sleep is common in patients with epilepsy. It can result in impairment of quality of life and day time functioning. Inadequate sleep can exacerbate the daytime drowsiness and memory dysfunctions, which are common in these patients, and can contribute to seizures becomes intractable one. There is a vicious cycle of sleep disruption, worsening of seizures, and further impairment of sleep that can be responsible for intractability in epilepsy.

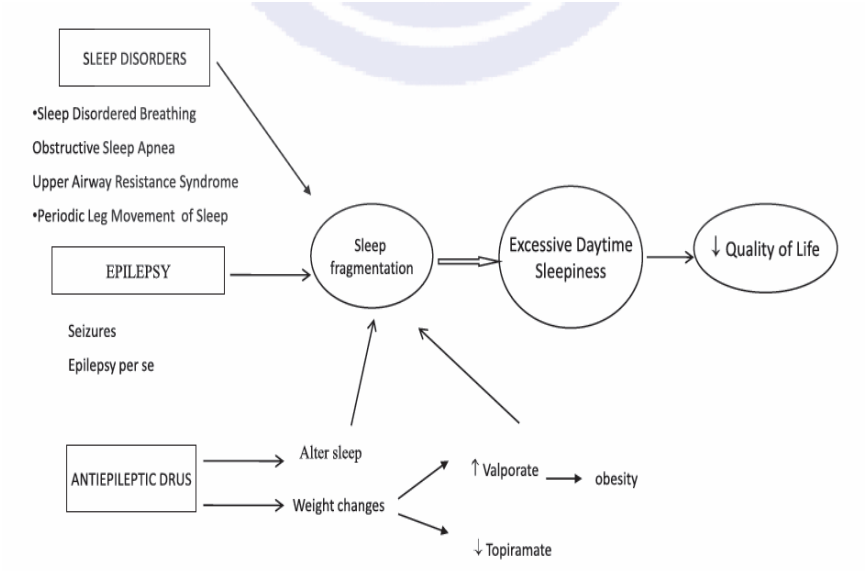
The causes of sleep disruption in epileptic patients are so many and include inadequate sleep hygiene, insufficient sleep, circadian rhythm disturbances, and co existing sleep disorders. In epilepsy, presence of epilepsy itself, and usage of anticonvulsant drugs can disrupt the sleep and even seizures occurring during wakefulness can disrupt the sleep.

Causes of sleep disruption in epilepsy:

Several studies have confirmed that sleep disorders and sleepiness are common epilepsy.

De Haas et al showed that patients with partial seizures have twice the prevalence of sleep disturbances than controls and the sleep disturbance is associated with further worsening of quality of life. This study also explains sleep disturbance in epileptic patients are not related to number of antiepileptic drugs being taken, suggesting that sleep disturbance may be inherent to the disorder itself.

Causes of sleep disruption in epilepsy:



EFFECT OF SLEEP ON EPILEPSY:

Relationship of sleep-wake state to epilepsy and IEDS (Inter-Ictal Epilepti form Discharges):

In 1885**, Gower**[35] found that 42% of patients had seizures solely in awake state, 21% of patients had seizures during sleep, and 37% had seizures during both sleep and wake. The amount of baseline rhythmicity occurring in the brain differs between the state of sleep and wake time.

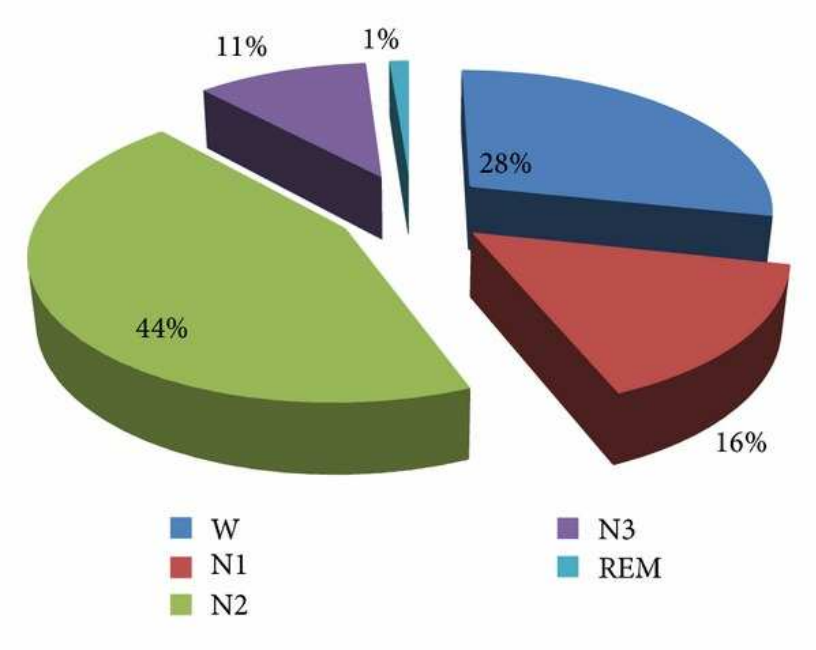
Sleep has a pronounced effect on secondary generalization of partial seizures. **IEDs are generally activated by NREM third and fourth stage** in focal epilepsy and **relatively suppressed by REM sleep**). **Malow et al.,** found that IEDs occurred frequently in deepened sleep (N3 sleep). Seizures in sleep also tend to occur during NREM sleep,) they are frequently observed in stage-2 NREM sleep, followed by stage-1 and then stage-3 and stage-4. **Minecin et** al., examined seizure rates in various stages of sleep in epilepsy patients undergoing overnight polysomnography. A total of 55 patients included in that study 95% of seizures occurred in NREM stage (14% stage-3&4, 20% stage-1, 61% stage-2) and only 5% of seizures in REM sleep. So from this they concluded that both IEDs and seizures are facilitated by NREM sleep.

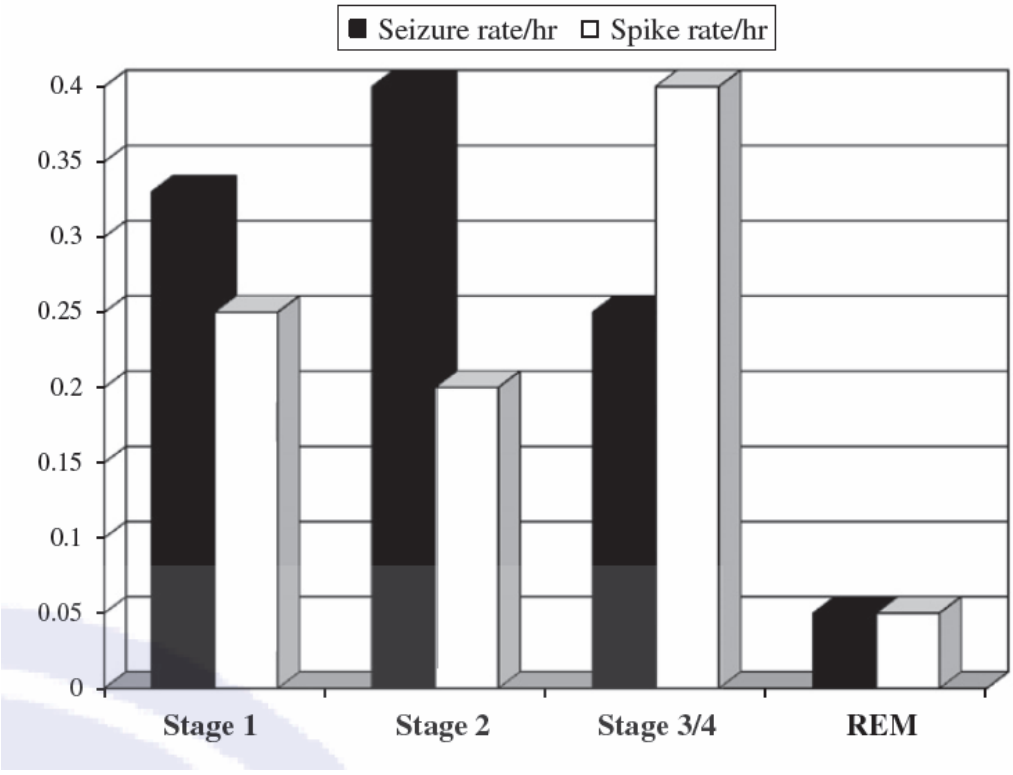
MECHANISMS INFLUENCING SLEEP AND EPILEPSY

NREM sleep represents a state of synchronization between thalamus and cortex and brain stem reticular activation system. The process of deepening of NREM sleep is correlated with reduction of effect of acetylcholine with progressive hyper polarization of neurons in the thalamocortical area. This hyper polarization in various levels may facilitate seizures and IEDs. REM sleep is characterized by inhibition of thalamocortical synchronyzing mechanism , inhibition of spread of epileptiform discharge, a desynchronized pattern of EEG, and skeletal muscle paralysis.

Sleep disturbances associated with epileptic patients are diagnosed by using polysomnography.

Distribution of seizure frequency during sleep:





Epileptiform discharges were noted mainly during stages 1 and 2 sleep, whereas they were scantily present in slow-wave sleep. No epileptiform discharges were seen during REM sleep.

Two state-specific components affecting epilepsy are the degree to which cellular discharge patterns are synchronized and alterations in antigravity muscle tone (**Shouse MN et al 1996)[36].**

REM sleep is also called paradoxical sleep because it is characterized by a highly active brain in a paralyzed body (**Carskadon MA et al** 2000)[37].

NREM sleep differs from REM sleep in that EEG activity is synchronized and postural muscle tone is decreased. REM sleep differs from NREM sleep in that EEG activity is desynchronized and also postural muscle tone is absent.

During NREM sleep, every cell in the brain discharges synchronously, and the discharge may even reach paroxysmal levels similar to those in epileptic states. Synchronous synaptic effects, whether, excitatory or inhibitory, are agument the magnitude and propagation of postsynaptic responses, including epileptic discharges. During REM sleep, cells discharge asynchronously. The divergent synaptic signals associated with asynchronous discharge

patterns are less likely to augment the propagation of epileptic EEG discharges.

Antigravity muscle tone is preserved in NREM sleep and waking, thus permitting seizure-associated and parasomnia-associated movement. But during REM sleep, profound lower motor neuron inhibition occur, it creats virtual paralysis (but diaphragm spared to permit continued respiration. Disruption of this component of REM sleep might underlie RBD and can influence clinically evident motor seizures.

SLEEP DISORDERS ASSOCIATED WITH PATIENTS WITH EPILEPSY:

Seizures may manifest as recurrent dreams, nightmares, or disorders of arousals such as sleep terrors and sleep walking. Autosomal dominant frontal epilepsy sometimes may also manifest as recurrent nightmares **(Scheffer IE et al 1995)[38].**

Common sleep disorders presents with epilepsy are excessive day time sleepiness; sleep disordered breathing, insomnia, nightmares, periodic limb movement disorder, disorders of arousal.

ROLE OF POLYSOMNOGRAPHY IN EPILEPSY:

The main cause for referral in epileptic patients is excessive day time sleepiness, suspected obstructive sleep apnoea (OSA), and characterization of nocturnal spells. The most common polysomnographic finding in epilepsy is OSA, although also found narcolepsy, insufficient sleep syndrome with possible idiopathic hypersomnolence, and previously unrecognized nocturnal seizures.

Beth A .Malow et al 1997[39], conducted a study about usefulness of polysomnography in epileptic patients found that some patients with OSA, treated with continuous positive airway pressure and treated with followup appointments. The majority of patients treated for OSA or other disorders have improvement in sleepiness or seizure control. It indicates the role polysomnography in epileptic patients.

AIM & OBJECTIVE OF THE STUDY

The primary aim of the study was to investigate the prevalence of sleep disturbances among generalized tonic-clonic patients and compare their polysomnography parameters with the normal controls and assessing their serum Ghrelin levels.

OBJECTIVES:

1. To assess the sleep changes and serum Ghrelin levels in patients with generalized tonic- clonic seizures.
2. To compare the serum Ghrelin levels in patients with generalized tonic clonic seizures and normal persons.
3. To correlate serum Ghrelin levels with sleep.

MATERIALS AND METHODS

The study was conducted during the year 2014 at the Institute of Physiology and Experimental Medicine, Madras Medical College after getting approval from Institutional Ethics Committee (IEC), Madras Medical College Chennai.

SELECTION OF SUBJECTS

Thirty patients with generalized tonic clonic seizures of age group between 20-40yrs will participate in the study and thirty age matched apparently healthy persons selected from community will be controls.

INCLUSION CRITERIA:

Both men and women in the age group of 20 – 40 yrs diagnosed with generalized tonic clonic seizures.

EXCLUSION CRITERIA:

Patient with the following conditions were excluded from the study:

- Children
- Presence of any concurrent psychiatric disease
- Conditions mimics epilepsy.

Other diseases (neurological, endocrinological, rheumatological, haematological, infectious disease, or inflammatory).

- Kidney or liver dysfunction
- Substance abuse
- Sleep disorders
- Controls were age matched healthy subjects with normal sleep habits.

With these criteria a total of 60 male and female individuals of were selected, Of this 30 were Generalized –tonic clonic patients and remaining 30 were healthy age matched controls. After thorough explanation of the study, informed verbal and written consent was obtained from the participants, and polysomnography (PSG) was conducted when patient is in stable condition.

STUDY DESIGN: cross sectional study

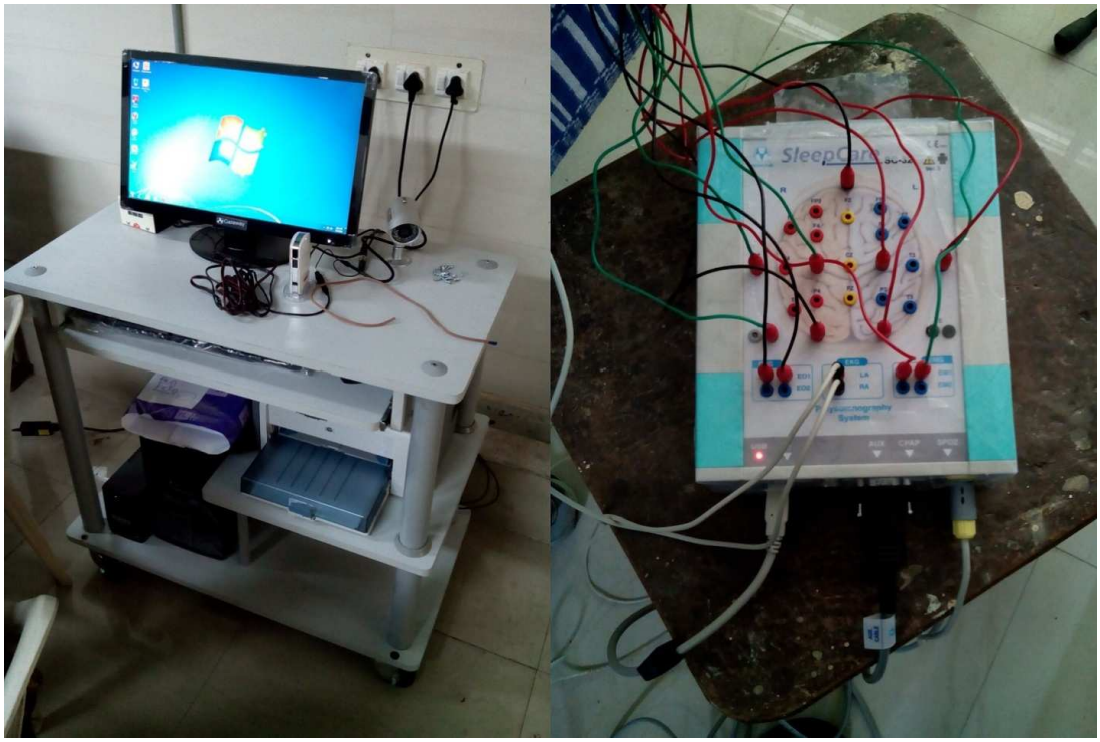
TYPE OF STUDY: comparative study

PLACE OF STUDY: Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai 03 and Institute of Neurology, Rajiv Gandhi Govt. General Hospital, Chennai-3.

REQUIREMENTS FOR CONDUCTING POLYSOMNOGRAPHY

- Air conditioned room with attached bathroom
- Polysomnographic recording system
- Computer
- Amplifiers
- Electrodes and application material
- Pulse oxymeter-to detect blood gas analysis
- Abdominal and thoracic belts-to detect respiratory effort
- Nasal airway pressure transducer- to detect nasal airflow
- Access to emergency medical care

Figure



PRESTUDY PROCEDURE:

- Detailed sleep wake history is taken
- Complete medical history and clinical examination should be completed
- Information is provided to the patient about the purpose and procedure of sleep study

Patient should be made aware that their sleep will be monitored throughout the entire study and they should be told how to contact the technologist if necessary.

THE PATIENT WAS ASKED TO FOLLOW THE INSTRUCTIONS:

- To take a bath in the evening and shave of facial hair
- Not to apply oil anywhere on the body
- To take dinner at least one hour before sleep study
- Not to consume alcohol on the day of study
- Avoid coffee or tea at least 3 hours before the study
- Dress in routine sleep wear
- Remove all ornaments
- To bring all previous medical reports
- Report for sleep study at the appointed time

PATIENT TRAY CONSISTS OF

- EEG paste
- Measuring tape
- Cotton swabs
- Electrodes, sensors, and lead wires
- Spirit
- Micropore
- Gloves
- Scissors
- Emergency anticonvulsant drugs.

DIGITAL SPECIFICATION FOR ROUTINE POLYSOMNOGRAPHY (AASM GUIDELINES)

Electrode	Desirable sample rate(Hz)	Minimal sampling rate (Hz)	High frequency filter (Hz)	Low frequency filter (Hz)	Maximum impedance (K Ohms)
EEG	500	200	35	0.3	5
EOG	500	200	35	0.3	5
EMG	500	200	100	10	
EKG	500	200	70	0.3	
Snoring	500	200	100	10	
Airflow	100	25			
Oximetry	25	10			
Chest and	100	25			

Electrode	Desirable sample rate(Hz)	Minimal sampling rate (Hz)	High frequency filter (Hz)	Low frequency filter (Hz)	Maximum impedance (K Ohms)
abdominal movement					
Body position	1	1			

Signal pathway and Processing in Polygraphic Circuit

Electrical signal produced in the patient's body

↓

Signal is transmitted from the skin to the electrode

↓

From the electrode through lead wire it goes to head box

↓

From head box it goes to differential amplifier through a shielded cable

↓

Differential amplifier subtracts a reference signal and a ground signal from the original signal using a method called COMMON MODE REJECTION

↓

The new signal is sent to polysomnograph

↓

DC devices send other data about the patient through DC amplifier to polysomnograph

SIGNAL MEASUREMENT

The recorded signal can be measured according to

a. Frequency- frequency is described as cycles per second or Hertz which refers to the number of waves appearing in the span of one second.
b. Amplitude – Amplitude refers to the vertical height of a wave and represents electrical voltage of the wave. It depends on the sensitivity setting of amplifier .

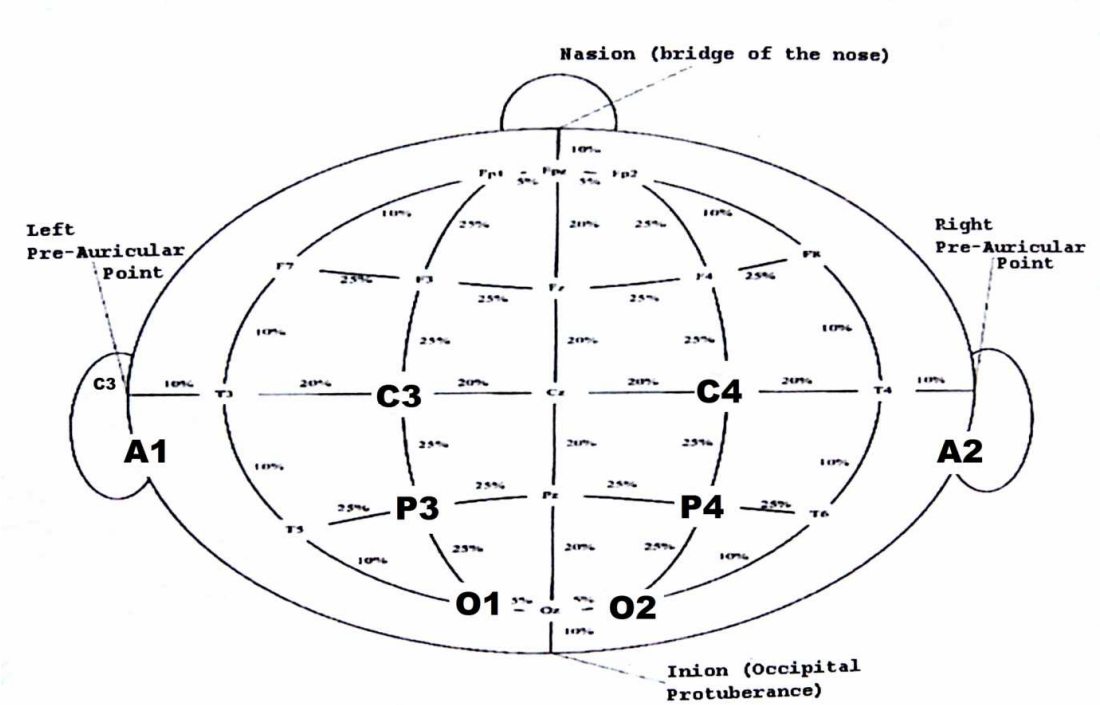
Sensitivity is defined as the amount of voltage necessary to produce a set deflection of the pen. Greater the sensitivity, lesser is the recorded amplitude.

SLEEP STUDY PROCEDURE:

Patient hookup: - It involves placement of various sensors to record the different parameters during sleep.

STEPS IN INTERNATIONAL 10-20 ELECTRODE PLACEMENT SYSTEM

International 10-20 system was developed as a standard measurement tool for placing electrodes on the head for recording EEG. The following electrode sites are located according to R&K [40] criteria-two mastoid (known as aural, A1 and A2), two central (C3 and C4) and two occipital derivations (O1&O2). EEG is recorded using either gold cup or silver chloride electrodes.



STEP IV

Location for four landmarks-nasion, inion, left preauricular region , and right preauricular region.

The distance from nasion to inion was measured. The 10 % of total distance from nasion is marked as Fpz and from inion as Oz along the line joining them. The halfway point between nasion and inion is marked as Cz. 20 % distance from Cz in front is marked as Fz and back as Pz.

The distance between left and right preauricular point was measure, with the tape passing through the halfway mark of Cz.

From the Cz 20 % of the distance on the left is marked as C3 and right as C4 on the line joining the left and right preauricular points.

The circumference of the head is measured by passing the through all the 10% marks. 50% of this measurement coincides with Oz at the back and Fpz in the front. 5% of circumference to the left of Oz is marked as O1and right as O2. Similarly 5% circumference to left of Fpz is marked as Fp1 and right as Fp2.

The distance from Fp1 to O1 passing through C3 is measured. 50%of this distance should intersect at C3. 25% of this distance is marked on the line joining Fp1and C3 as F3 and similarly on the right side as F4.

A1 and A2 are placed behind the ears on the mastoid process.

For recording the electro oculogram, the placement of an electrode is located 1 cm out and below the outer canthus of the left eye (E1) and another electrode placed 1 cm out and above the outer canthus of right eye (E2) with reference to the right mastoid process (A2). It records the retino- corneal potential difference .

For recording the electromyogram of the chin ,two electrodes are placed just below the chin , one electrode placed 2cm below and 2cm left of midline and the others is 2cm below and 2cm to the right of midline.

Respiratory effort belts are placed snugly around the thorax and abdomen.

Airflow sensors- Nasal prongs connected to a pressure transducer is used for measuring nasal flow (gives an indirect measure of flow based on the recording of the pressure at the gives an indirect measure of flow based on the recording of the pressure at the prongs relative to the atmospheric pressure.)

Oximeter sensor is placed on any of the three middle fingers . it is a noninvasive method for monitoring the hemoglobin percentage saturated with oxygen. Snore sensor is also connected.

Leg EMG leads records activity of anterior tibialis muscle. Electrodes are placed on the outer aspect of lower half of each leg.

PARAMETERS RECORDED:

- ELECTROENCEPHALOGRAM
- CHIN ELECTROMYOGRAM

- ELECTRO OCULOGRAM
- ELECTROCARDIOGRAM
- AIRFLOW
- ABDOMINAL AND THORACIC RESPIRATORY EFFORT
- LEG/ARM ELECTROMYOGRAM
- SATURATION
- SNORING
- BODY POSITION
- HEART RATE

After all sensors are applied start the study and check the impedance and signal of all the electrodes. Perform biocalibration – at the start of the study before lights out and just after lights on at the end of the study.

Close eyes-instruct patient to lie down with eyes closed for 30 seconds. This helps to reveal the alpha activity.

Open eyes-instruct patient to lie still down with eyes open for 30 seconds. This helps to eliminate the alpha activity.

Look left and right- instruct patient to look to left and right repeatedly while holding the head still. This mimics the eye movement seen during REM sleep

Look up and down- instruct patient to look to the up and down repeatedly while holding the head still. This differentiates the vertical and horizontal eye movements.

Hold breath-Instruct the patient to take a deep breath and hold it for 5-10 seconds. This mimics the central apnoea

Respiratory effort- Instruct he patient to move the chest and abdomen in and out while holding breath. This mimics an obstructive apnoea.

Move feet- Instruct the patient to move the feet. This mimics leg movements during sleep.

During the study the observer should document

Time of sleep study

- Biocalibration
- Technical difficulties and methods of correction
- Patient complaints

Post study procedure

- Ensure that study is saved properly
- Clean and sterilize the electrodes and various sensors

SCORING AND DATA ANALYSIS IS DONE TO RECOGNIZE THE FOLLOWING EVENTS

- Sleep staging
- Arousal
- Cardiac events
- Respiratory events
- Movement events

SCORING BY EPOCHS

The polygraph record is divided into segments of equal size, with epoch length of 300mm and duration of 30 seconds.

A single stage score is assigned to each epoch-when more than one stage is present in each epoch-the stage score of the epoch is determined by the stage that takes up the greatest portion of the epoch.

SLEEP STAGES SCORING:

Sleep stage are scored at 30 sec sequential recordings known as epochs.

Wake – A>50% of an epoch has alpha EEG waves over the occipital region with eye closure or if alpha waves absent the presence of any of the following Eye blinks (0.5-2Hz)

Reading eye movement which consists of slow conjugate movement followed by a rapid movement in the opposite direction, Rapid open eye movements associated with normal or high chin tone.

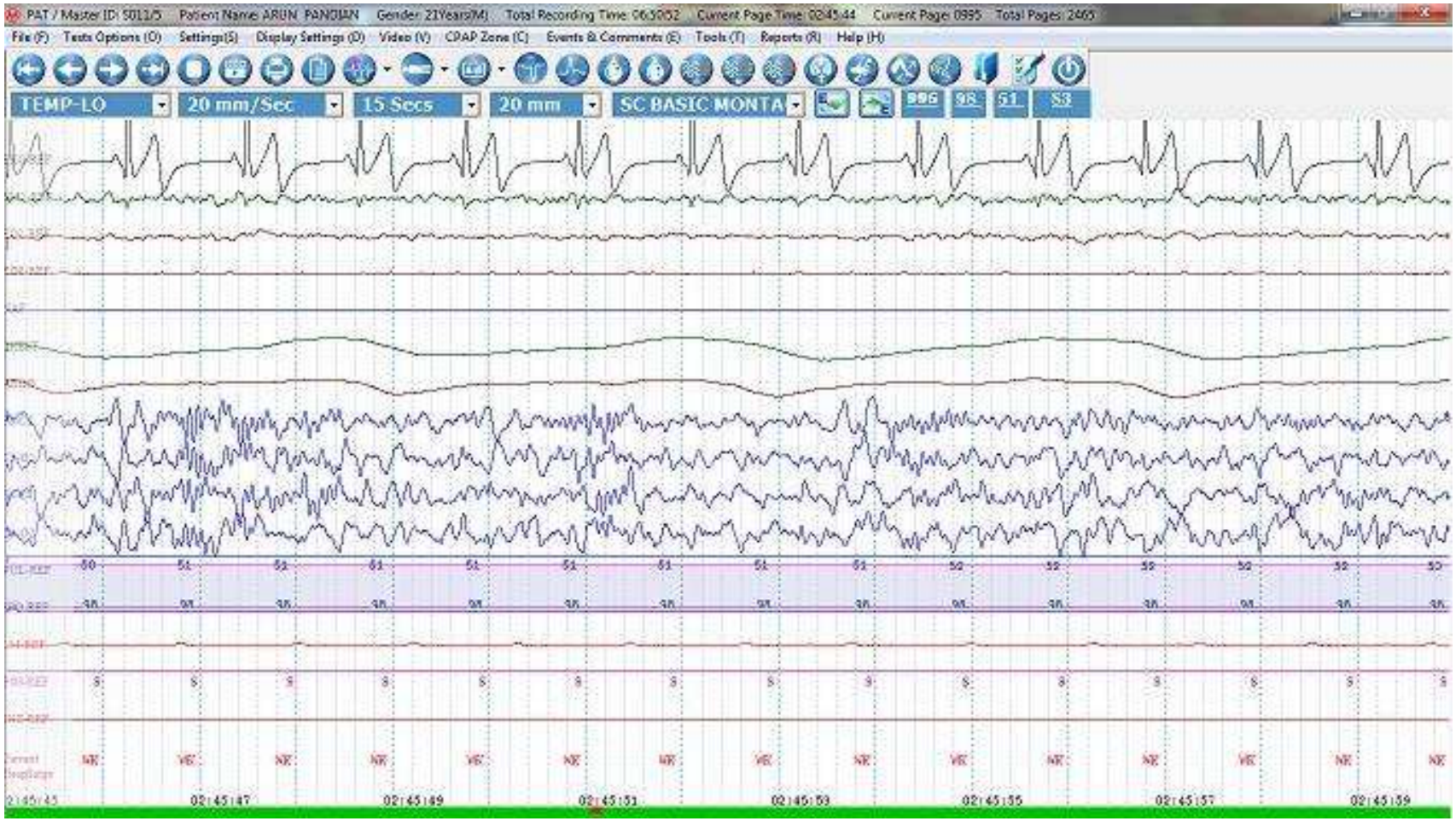


Figure Epoch of awake stage

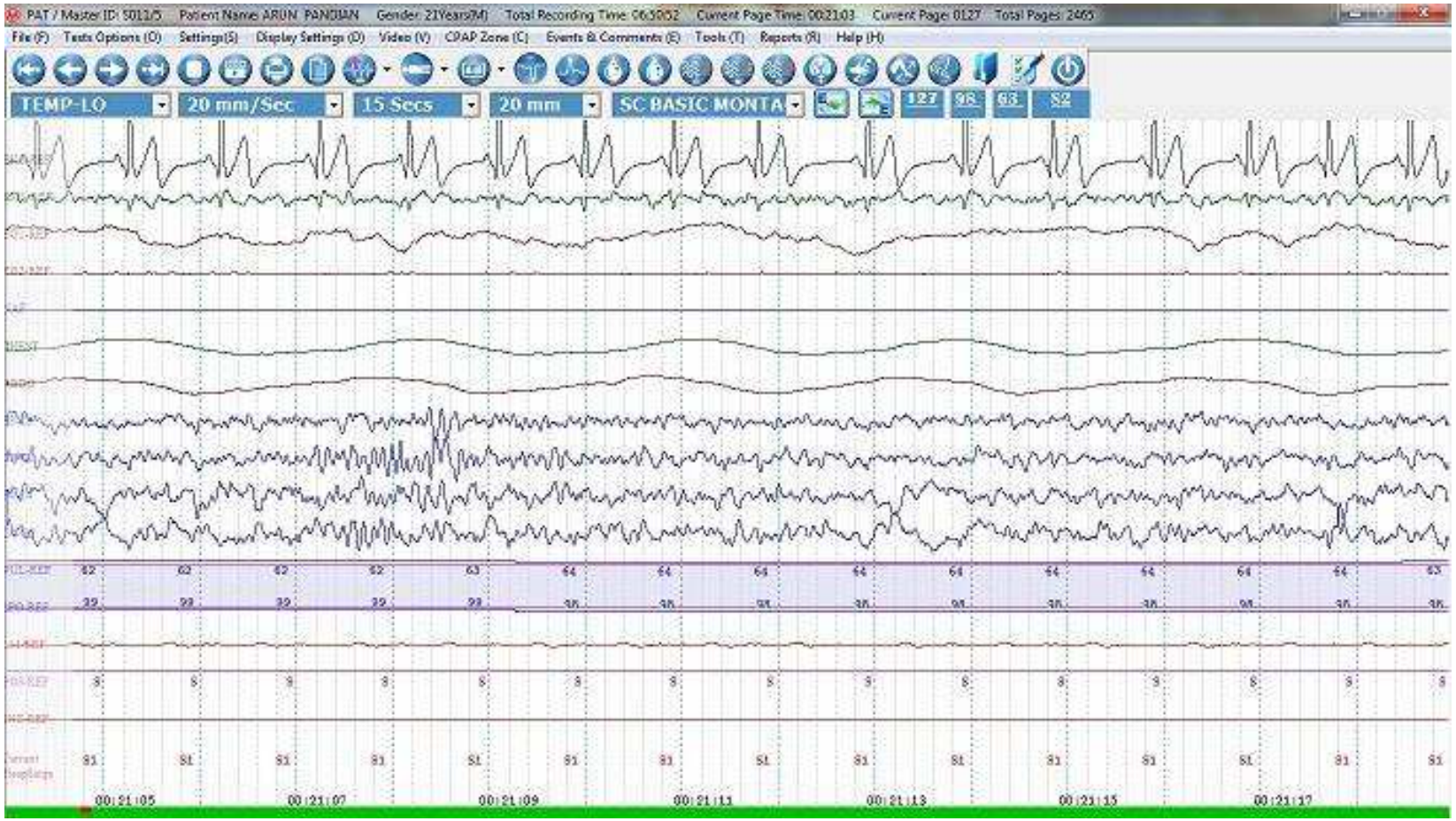


Figure Epoch of stage N1

Figure Epoch of stage N2

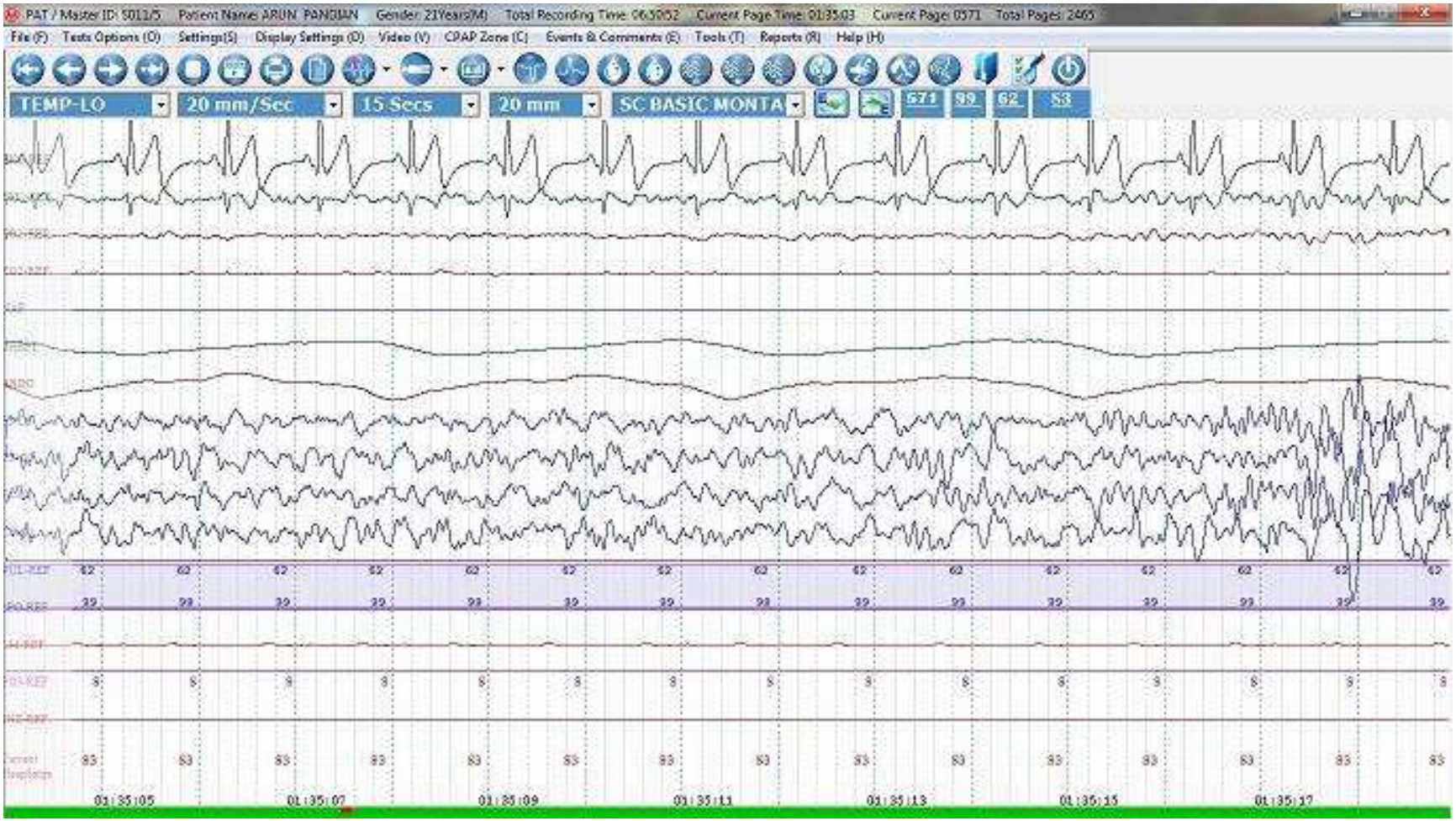


Figure Epoch of stage N3

Figure Epoch of stage REM

Stage-I- alpha waves being replaced by a low amplitude , mixed frequency (4 to 7Hz) waves occupying >50% of the epoch, or the presence of (for those who do not generate alpha waves)

Vertex sharp waves of <0.5 seconds duration that are maximal over central region

Slow eye movement

Stage II- defined by the presence of K complexes or sleep spindles.

Stage III and IV – indicated when >=20%of the epoch shows slow wave (0.5-2Hz and >75uV) EEG activity.

REM – presence of low amplitude, mixed frequency EEG activity, rapid eye movement on EOG, and low tone on EMG.

RESPIRATORY EVENTS SCORING RULES:

APNOEA-decrease in airflow amplitude by => 90% from baseline for duration of minimum10 seconds. It is scored as Obstructive-If inspiratory effort is present throughout the entire period

Central -If the inspiratory effort is absent throughout the period

Mixed –If inspiratory effort in the initial part of the period is followed by the presence of inspiratory effort.

Hypoapnoea-decrease in airflow amplitude by =>30% of baseline for a duration of at least 10 seconds accompanied by =>4% of oxygen saturation.

Arousal

During NREM sleep – abrupt frequency shift (eg.alpha , theta or frequencies >16 Hz) lasting =>3 seconds and preceded by =>10 seconds of stable sleep.

During REM sleep abrupt EEG frequency shift (e.g alpha, theta, or frequencies >16 Hz) lasting =>3 seconds and preceded by =>10 seconds of stable sleep, accompanied by an increase in chin EMG that is =>1 second duration.

Respiratory Effort Related Arousal (RERA)- breaths associated with heightened respiratory efforts or flat airflow waveform with a duration =>10 seconds and preceding an arousal but not meeting criteria for either apnoea or Hypoapnoea.

Periodic limb movement in sleep

Four consecutive leg movements (each 0.5 to 10 seconds in duration with an amplitude 8uV above resting EMG) characterized by period lengths of between 5 and 90 seconds between onset of consecutive movements.

Leg movements on different legs are counted as one movement if they are separated by <5 seconds between movement onsets.

Cardiac events scoring rules

- Asystole-cardiac pause >3 seconds
- Bradycardia- heart rate <40 beats per minute
- Sinus tachycardia-heart rate>90 beats per minute

SLEEP ARCHITECHTURE DEFINITION AND FORMULAE

- % Stage I = Minutes of Stage I *100/ Total Sleep Time (TST)
- % Stage II = Minutes of Stage II *100/ Total Sleep Time (TST)
- % Stage III & IV = Minutes of Stage III&IV *100/ Total Sleep Time (TST)
- % Stage R = Minutes of Stage R *100/ Total Sleep Time (TST)
- % Wake time=Minutes of Wake *100/ Sleep Period Time (SPT)
- Sleep Onset = the first three consecutive Epochs of Stage I sleep
- Latency to REM= the period of time from sleep onset to first Epoch of REM
- NAP onset (Sleep) latency= the period of time to lights out to sleep onset
- Sleep Period Time (SPT) = the time from sleep onset to last Epoch of sleep
- Total Recording Time (TRT) - The time from lights out to lights on

- Total Sleep Time (TST) - The amount of sleep recorded during TRT
- Wakefulness After Sleep Onset (WASO) - The Wakefulness occurring from sleep onset to last Epoch of sleep

OTHER INDICES

Apnoea Hypoapnoea Index (AHI) - Total number of apnoeas and Hypoapnoeas occurring per hour of sleep

Respiratory Disturbances Index (RDI)- Total number of apnoeas and Hypoapnoeas and Respiratory Effort Related Arousals occurring per hour of sleep

Desaturation Index (DI)- Total number of desaturation events per hour of sleep

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

INSTRUCTIONS: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

   USUAL BED TIME _____

2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, when have you usually gotten up in the morning?

   USUAL GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)

   HOURS OF SLEEP PER NIGHT _____

INSTRUCTIONS: For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...cannot get to sleep within 30 minutes	☐	☐	☐	☐
(b) ...wake up in the middle of the night or early morning	☐	☐	☐	☐
(c) ...have to get up to use the bathroom	☐	☐	☐	☐
(d ...cannot breathe comfortably	☐	☐	☐	☐
(e) ...cough or snore loudly	☐	☐	☐	☐
(f) ...feel too cold	☐	☐	☐	☐
(g) ...feel too hot	☐	☐	☐	☐
(h) ...had bad dreams	☐	☐	☐	☐
(i) ...have pain	☐	☐	☐	☐
(j) Other reason(s), please describe _____ _____				
How often during the past month have you had trouble sleeping because of this?	☐	☐	☐	☐

	Very good	Fairly good	Fairly bad	very bad
6. During the past month, how would you rate your sleep quality overall?	☐	☐	☐	☐

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	☐	☐	☐	☐
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	☐	☐	☐	☐

	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

	No bed partner or roommate	Partner/ roommate in other room	Partner in same room, but not same bed	Partner in same bed
10. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...loud snoring	☐	☐	☐	☐
(b) ...long pauses between breaths while asleep	☐	☐	☐	☐
(c) ...legs twitching or jerking while you sleep	☐	☐	☐	☐
(d) ...episodes of disorientation or confusion during sleep	☐	☐	☐	☐
(e) Other restlessness while you sleep; please describe	☐	☐	☐	☐

_____

_____

Epworth Sleepiness Scale

Name: _____

Date: _____

Your age: (Yr) _____ **Your sex:** ❑ Male ❑ Female

How likely are you to doze off or fall asleep in the situations described below, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the moit appropriate number for each situation:-

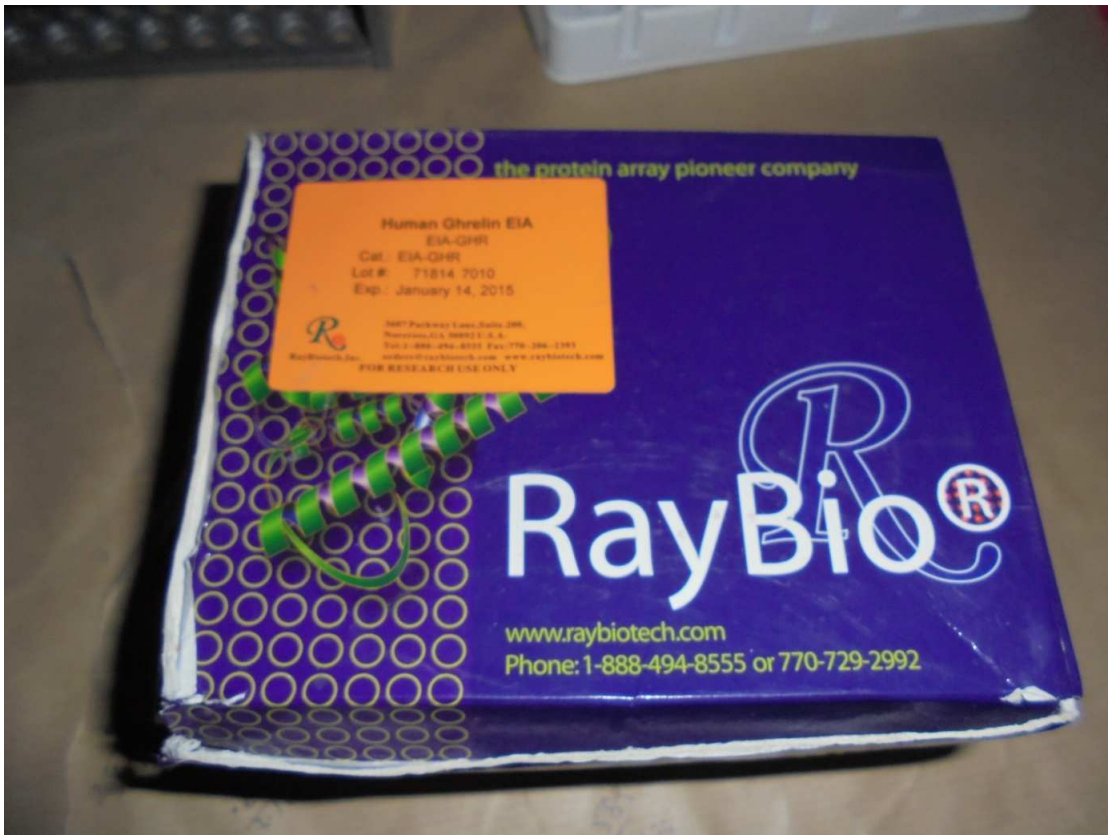
0 = would never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

Situation	Chance of dozing
Sitting and reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	
Total	

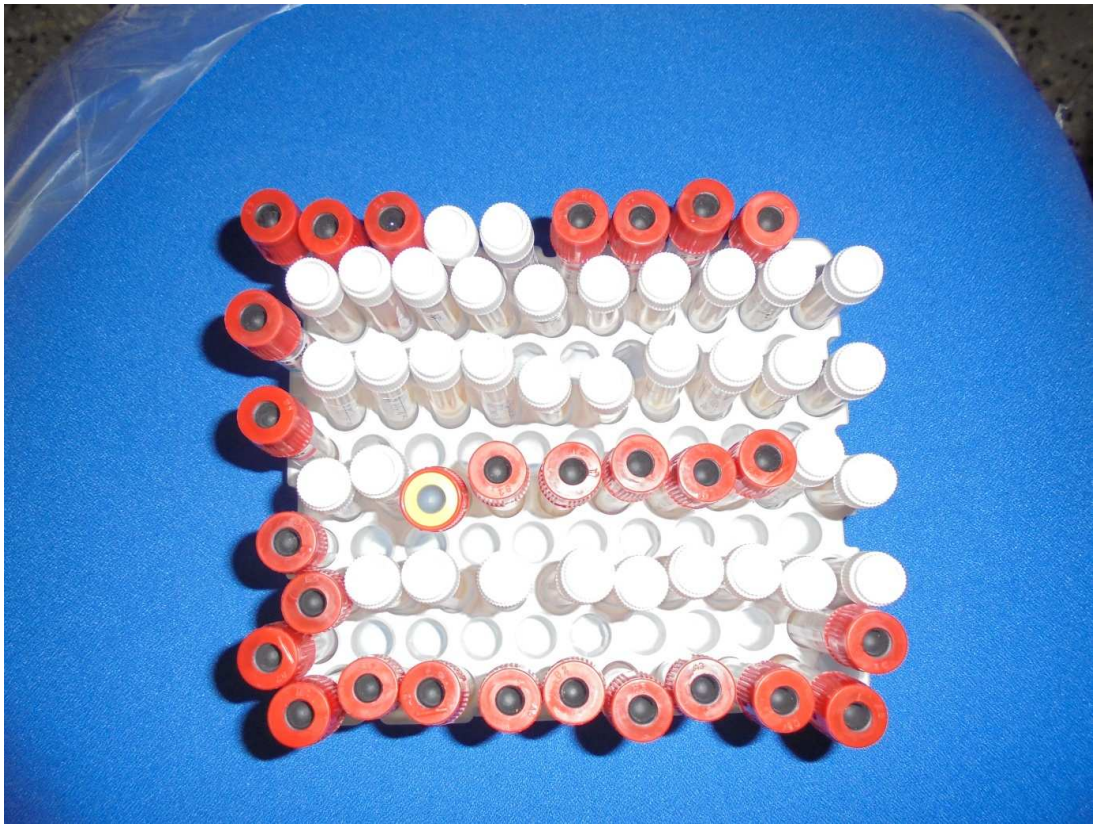
Score:	
0-10	Normal range
10-12	Borderline
12-24	Abnormal

4.3.2 STORAGE OF SAMPLES

A well maintained deep freezer with -20 degree c was available in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai. All the samples were stored in the deep freezer until estimation of serum ghrelin.




1 2 3 4 5 6 7 8
A
B
C
D
E
F
G
H



ESTIMATION SERUM GHRELIN:

Estimation was done using Human Ghrelin ELISA kit

Contents of the kit

1. Wash buffer
2. Lyophilized anti-Ghrelin polyclonal antibody
3. Standard
4. TMB substrate
5. Stop solution
6. Sample diluent

ASSAY PROCEDURE:

a. Preparation of reagents, samples and standards done.

b. Add 100 µl anti-Ghrelin antibodies to each well. Incubate 1.5 hours at room temperature.

c. Next add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature.

d. Then add 100 µl prepared streptavidin solution. Incubate 45 minutes at room temperature.

e. Then add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

f. Then add 50 µl Stop Solution to each well. Read at 450 nm immediately

CALCULATION OF RESULTS:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using Sigma Plot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance on the y-axis. Draw the best-fit curve through the standard points. (Ray Bio Human Ghrelin EIA Kit Protocol)

- Percentage absorbance = (B – blank OD)/ (Bo – blank OD)

  where

- B = OD of sample or standard and

- Bo = OD of zero standard (total binding

RESULTS

The data obtained from conducting the polysomnography and serum ghrelin were statistically analysed using the statistical package for social sciences (SPSS) software version 21. From the data, mean and standard deviation of the variables are determined for the individual groups. Student t testswas employed for statistical analysis.

- *P value < 0.05 was considered as significant.
- *P value < 0.01 was considered as highly significant
- *P value < 0.001 was considered as very highly significant.

COMPARISON OF AGE AND BMI:

In the two study groups, age is compared and the results shown in the table 5.1. The mean age of control group is 30.03±6.26, and that of GTCS group is 31.10±5.60. The difference between the age group is found to be statistically not significant (p value 0.4), and mean BMI of control group is 26.30±1.46 and that of GTCS group is 26.89±0.99. The BMI was found to be statistically not significant (p value 0.06).

TABLE I: Comparison of parameters between Control and GTCS patients

Variable	Group	N	Mean
Age	GTCS	30	31.10±5.60
	CONTROL	30	30.03±6.26
BMI	GTCS	30	26.89±0.99
	CONTROL	30	26.30±1.46

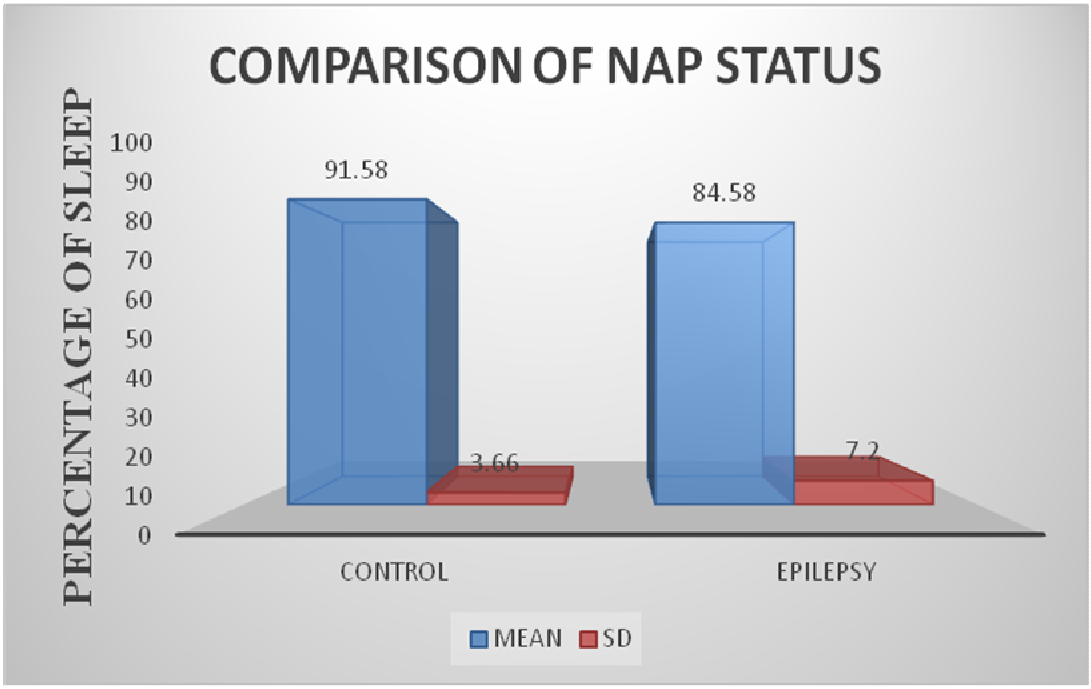
COMPARISON OF SLEEP EFFICIENCY/ NAP STATUS:

The NAP status percentage of study groups was measured and statistically analysed and the results are shown in table 5.2.

TABLE II: Comparison of Sleep Efficiency/ NAP status between Control and GTCS patients

Variable	Group	N	Mean	SD	P –Value
SLEEP EFFECIENCY %	Control	30	91.58	3.66	<0.000*
	GTCS	30	84.58	7.20	
P – Value < 0.001 very highly significant.					

The NAP status percentage of GTCS patients shows a very highly significant decrease (** p value < 0.000) when compared to control groups.



COMPARISON OF SLEEP STAGE I:

TABLE III: Comparison of Sleep Stage I between Control and GTCS patients

Variable	Group	N	Mean	SD	P –Value
Sleep stage I (Minutes)	Control	30	14.6	2.60	<0.000*
	GTCS	30	18.35	2.85	
* P – Value < 0.001 very highly Significant					

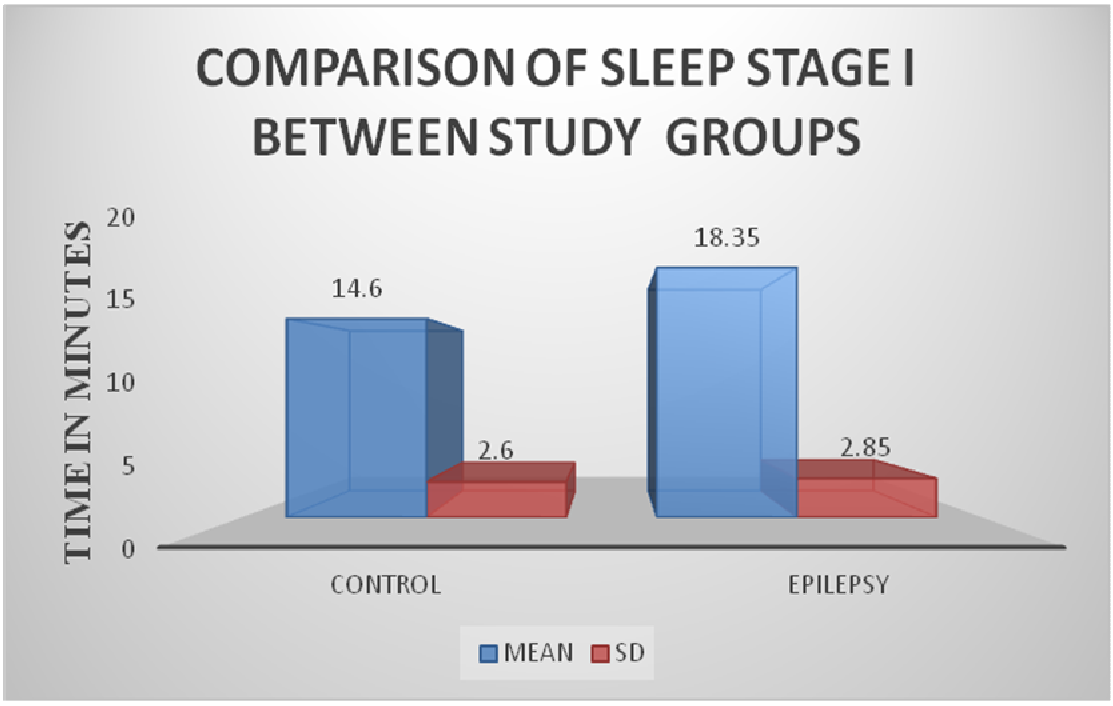
The difference between the sleep stage I duration in study groups is statistically very highly significant.

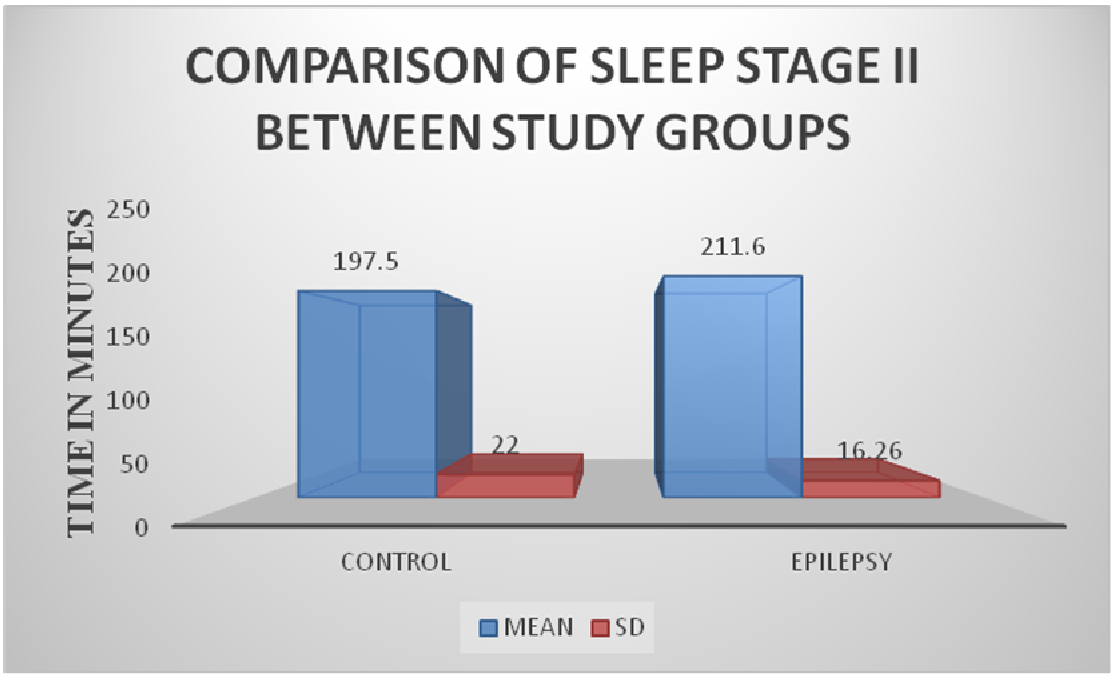
COMPARISON OF SLEEP STAGE II:

TABLE IV: Comparison of Sleep Stage II between Control and GTCS patients

Variable	Group	N	Mean	SD	P -Value
Sleep stage II (Minutes)	Control	30	197.5	22	<0.006*
	GTCS	30	211.6	16.26	
* P – Value < 0.001 is very highly Significant					

The difference between the sleep stage II duration in study groups is statistically very highly significant.


COMPARISON OF SLEEP STAGE I
BETWEEN STUDY GROUPS
TIME IN MINUTES
20
15
10
5
0
14.6
2.6
18.35
2.85
CONTROL
EPILEPSY
MEAN
SD


COMPARISON OF SLEEP STAGE II
BETWEEN STUDY GROUPS
TIME IN MINUTES
250
200
150
100
50
0
197.5
22
211.6
16.26
CONTROL
EPILEPSY
MEAN
SD

COMPARISON OF SLEEP STAGE III:

TABLE V: Comparison of Sleep Stage III between Control and GTCS patients

Variable	Group	N	Mean	SD	P –Value
Sleep stage III (Minutes)	Control	30	40.9	7.20	<0.042*
	GTCS	30	36.98	7.40	
* P – Value < 0.05 is Significant.					

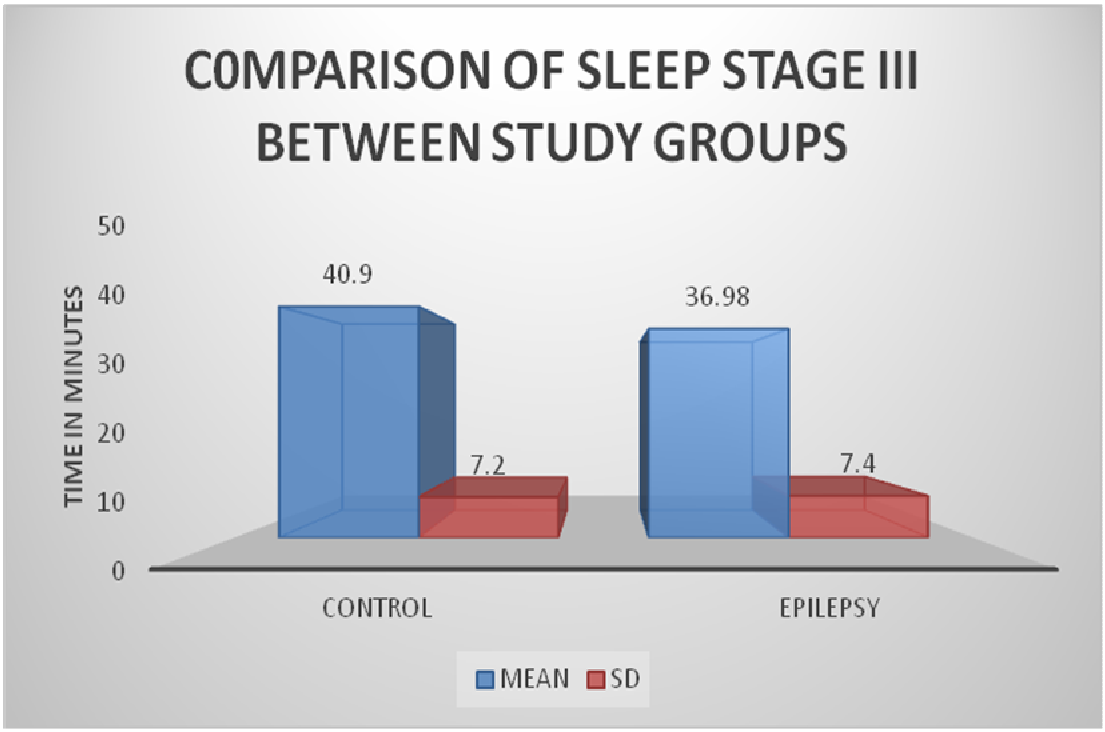
The sleep stage III of GTCS group shows a significant decrease (p value 0.042) when compared to control group.

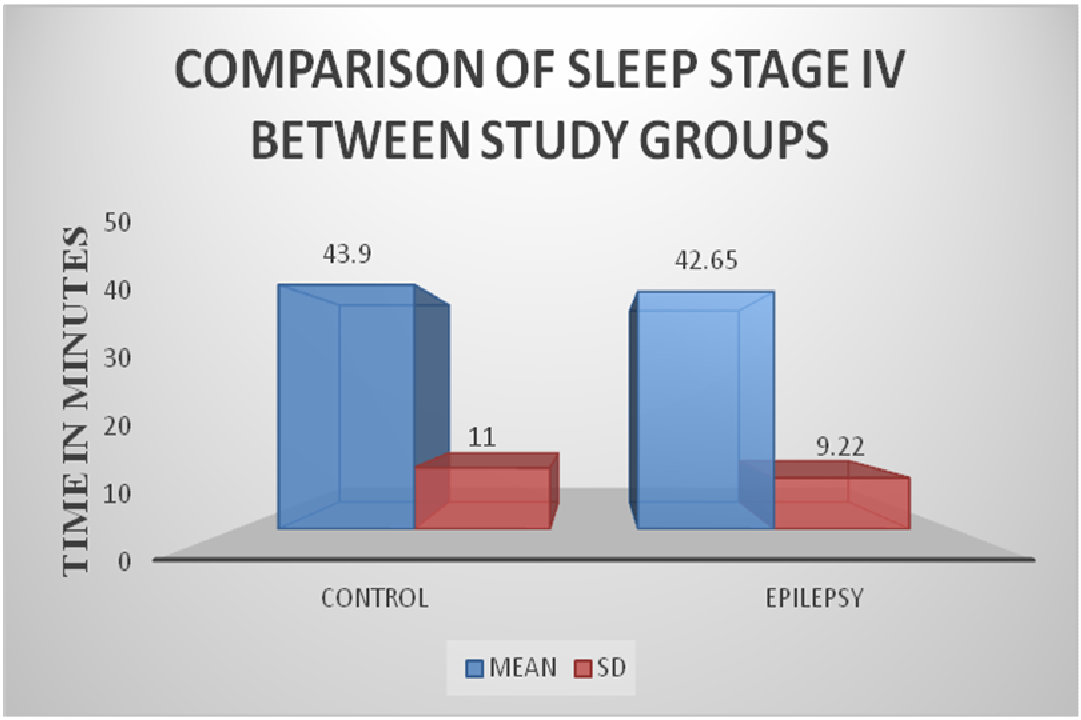
COMPARISON OF SLEEP STAGE IV:

TABLE VI: Comparison of Sleep Stage IV between Control and GTCS patients

Variable	Group	N	Mean	SD	P –Value
Sleep stage IV (Minutes)	Control	30	43.9	11	<0.6
	GTCS	30	42.65	9.22	
* P – Value < 0.05 Significant					

The sleep stage IV of GTCS group shows a decrease but not a significant decrease (p value 0.042) when compared to control group.





COMPARISON OF REM SLEEP STAGE:

TABLE VII: Comparison of REM Sleep Stage between Control and GTCS patients

Variable	Group	N	Mean	SD	P – Value
REM Sleep Stage (Minutes)	Control	30	77.27	13.3	<0.000*
	GTCS	30	63.93	13.21	
** P – Value < 0.001 very highly Significant					

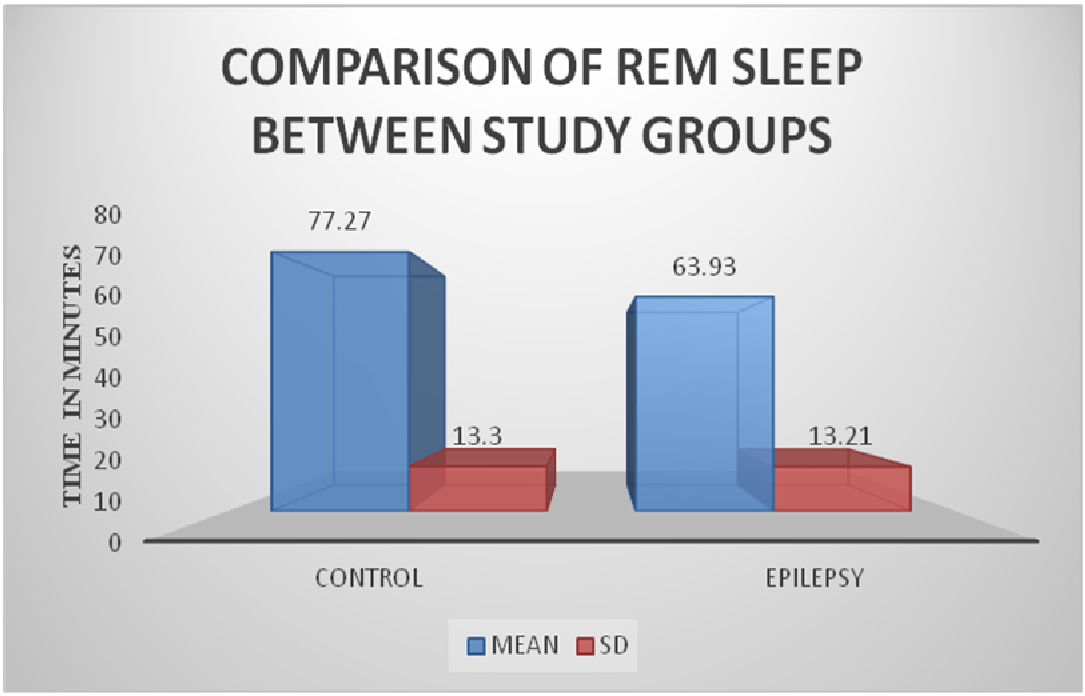
The REM sleep stage of GTCS group shows a significant decrease (p value< 0.000) when compared to control group.

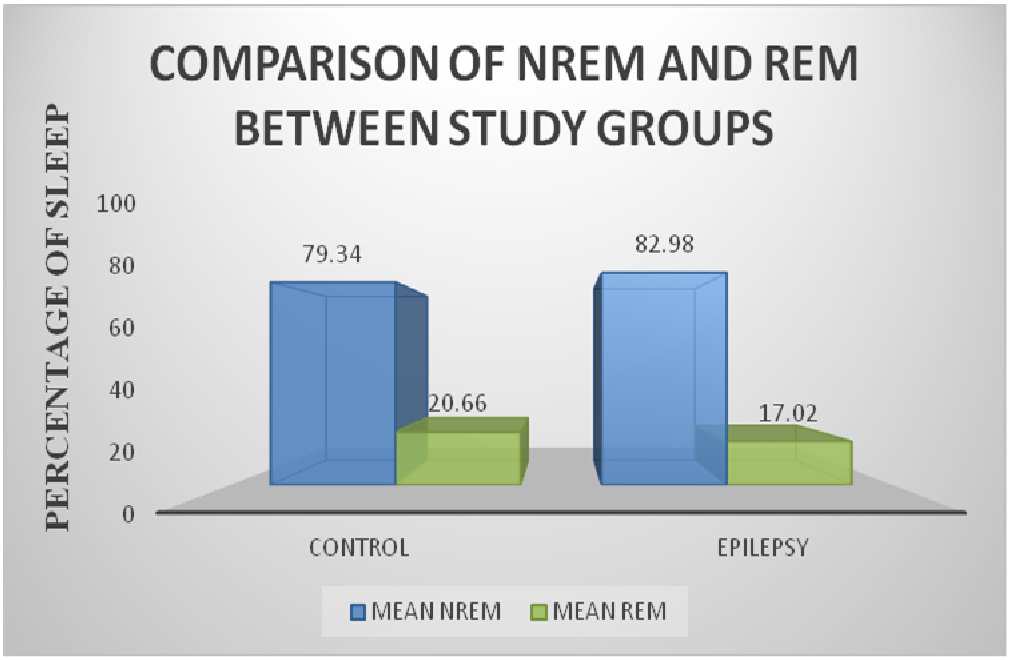
COMPARISON OF NREM SLEEP:

TABLE VIII: Comparison of NREM sleep between Control and GTCS patients

Variable	Group	N	Mean	SD	P – Value
NREM sleep (Minutes)	Control	30	296.8	69.2	<0.3
	GTCS	30	309.52	20.5	
* P – Value <0.05 is Significant					

The difference between the NREM sleep stage is in between study groups is statistically not significant.





COMPARISON OF SLEEP STAGE PERCENTAGE:

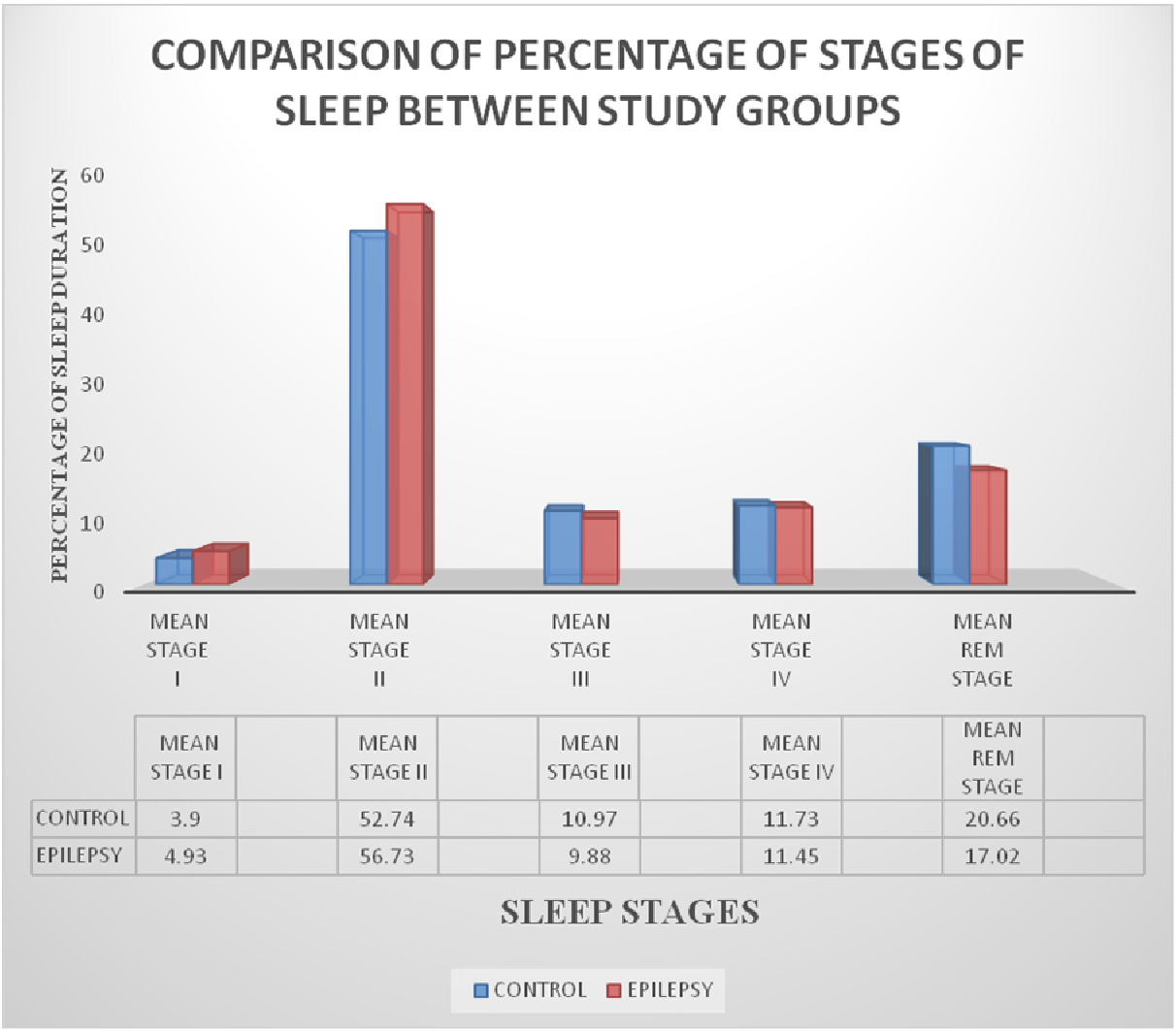
TABLE IX: Comparison of Sleep Stage Percentage between Control and GTCS patients

Variable	Group	Mean	SD	P – Value
Stage I % (Stage I/Total Sleep Time*100)	Control	3.90	0.66	<0.000*
	GTCS	4.92	0.68	
Stage II % (Stage II/Total Sleep Time*100)	Control	52.74	4.12	<0.000*
	GTCS	56.73	3.13	
Stage III % (Stage III /Total Sleep Time*100)	Control	10.97	1.88	<0.02*
	GTCS	9.88	1.90	

Variable	Group	Mean	SD	P – Value
Stage IV % (Stage IV/Total Sleep Time*100)	Control	11.73	2.61	<0.67
	GTCS	11.45	2.51	
REM % (REM/Total Sleep Time*100)	Control	20.66	3.20	<0.000*
	GTCS	17.02	2.78	

* P – Value < 0.05 Significant

** P – Value < 0.001 Very Highly Significant



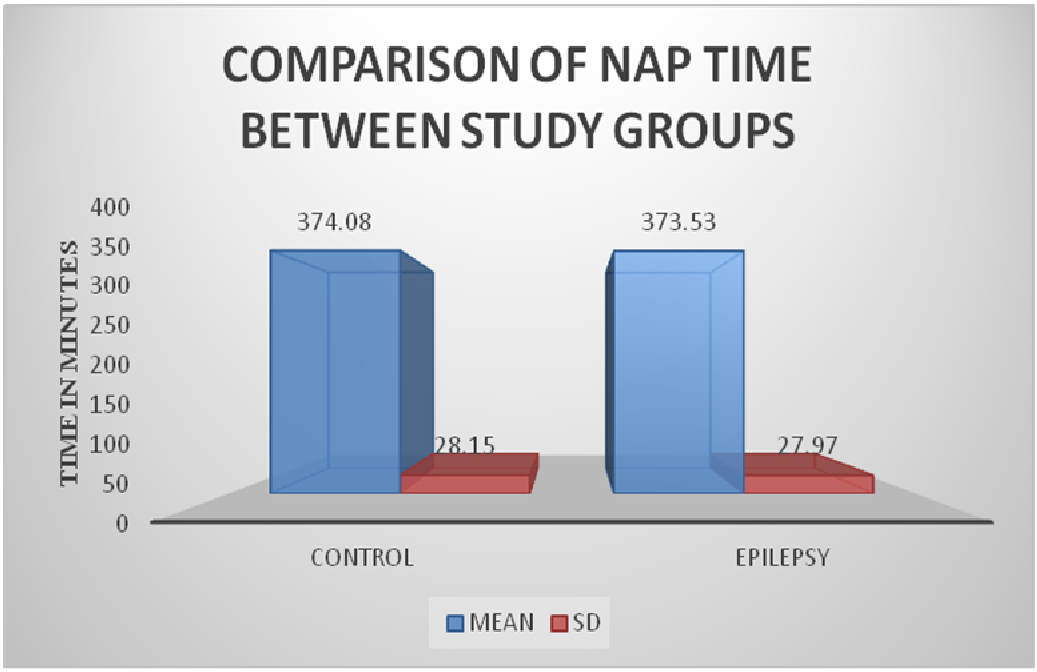
	MEAN STAGE I	MEAN STAGE II	MEAN STAGE III	MEAN STAGE IV	MEAN REM STAGE
CONTROL	3.9	52.74	10.97	11.73	20.66
EPILEPSY	4.93	56.73	9.88	11.45	17.02

COMPARISON OF TOTAL SLEEP TIME/ NAP TIME:

TABLE X: Comparison of Total sleep time/ Nap time Control and GTCS patients

Variable	Group	N	Mean	SD	P – Value
Total sleep time (Minutes)	Control	30	374.08	28.15	<0.93
	GTCS	30	373.53	27.97	
* P – Value < 0.05 Significant					

The NAP time of GTCS group shows a decrease, but not a significant (p value< 0.93) when compared to control group.

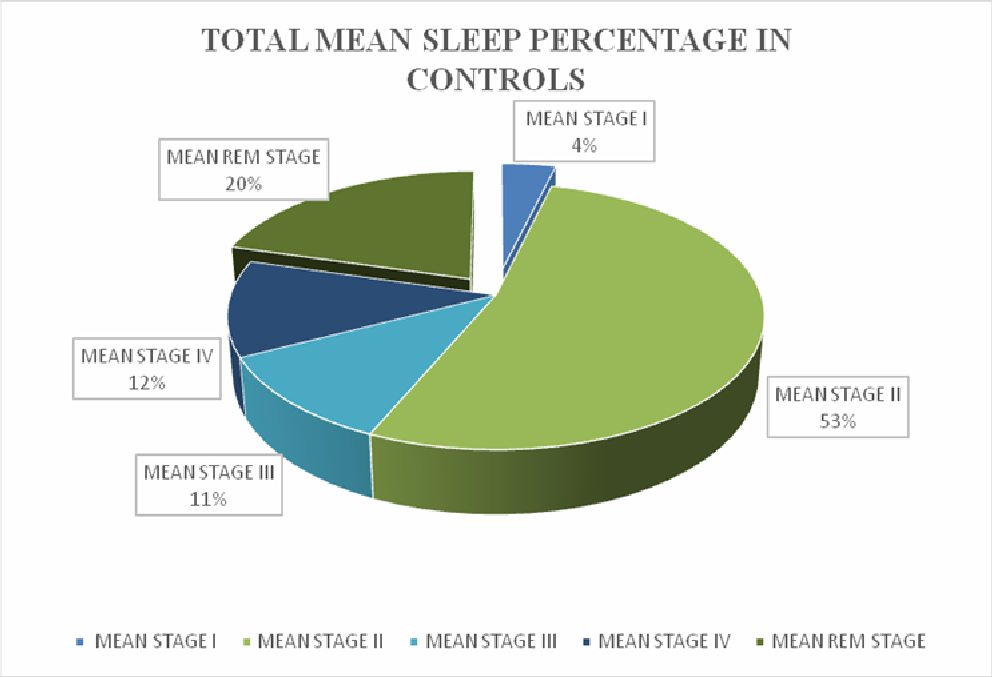


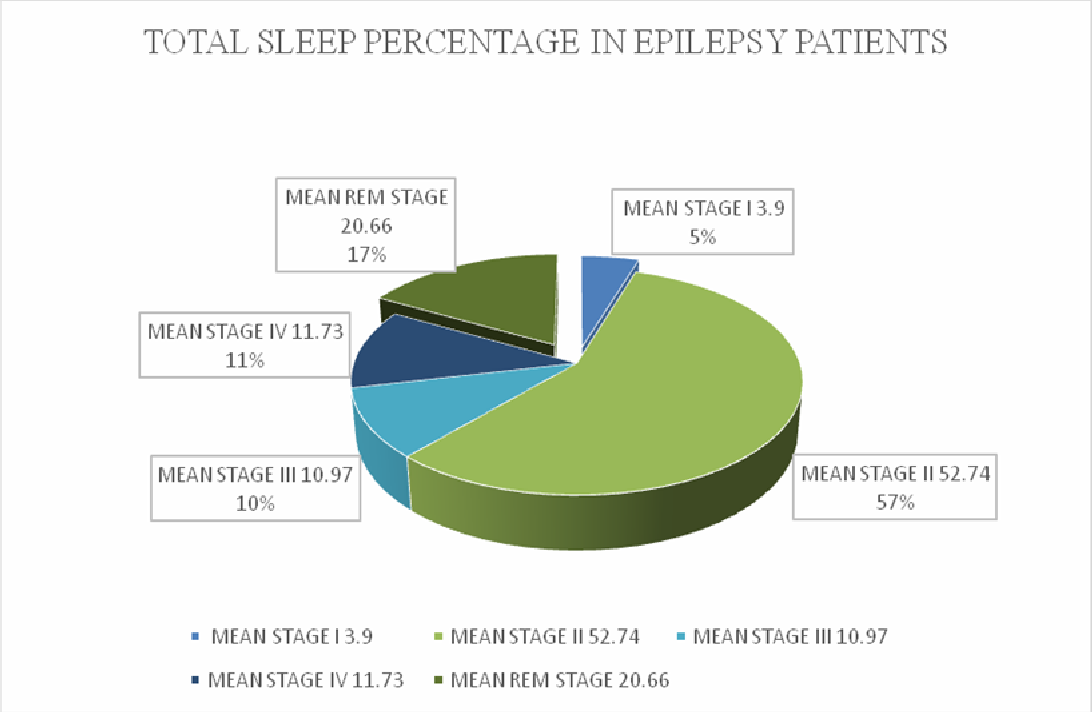
COMPARISON OF NAP ONSET LATENCY:

TABLE XI: Comparison of NAP onset latency between Control and GTCS patients

Variable	Group	N	Mean	SD	P – Value
NAP onset latency (Minutes)	Control	30	14.37	5.9	<0.4
	GTCS	30	15.52	5.9	
* P – Value < 0.05 Significant					

The NAP onset latency of GTCS group shows a decrease, but not a significant (p value< 0.4) when compared to control group.


TOTAL MEAN SLEEP PERCENTAGE IN CONTROLS
MEAN STAGE I 4%
MEAN REM STAGE 20%
MEAN STAGE IV 12%
MEAN STAGE II 53%
MEAN STAGE III 11%
MEAN STAGE I
MEAN STAGE II
MEAN STAGE III
MEAN STAGE IV
MEAN REM STAGE


TOTAL SLEEP PERCENTAGE IN EPILEPSY PATIENTS
MEAN REM STAGE 20.66 17%
MEAN STAGE I 3.9 5%
MEAN STAGE IV 11.73 11%
MEAN STAGE III 10.97 10%
MEAN STAGE II 52.74 57%
MEAN STAGE I 3.9
MEAN STAGE II 52.74
MEAN STAGE III 10.97
MEAN STAGE IV 11.73
MEAN REM STAGE 20.66

COMPARISON OF SUBJECTIVE SLEEP SCORES:

TABLE XII: Comparison of subjective sleep scores between Control and GTCS patients

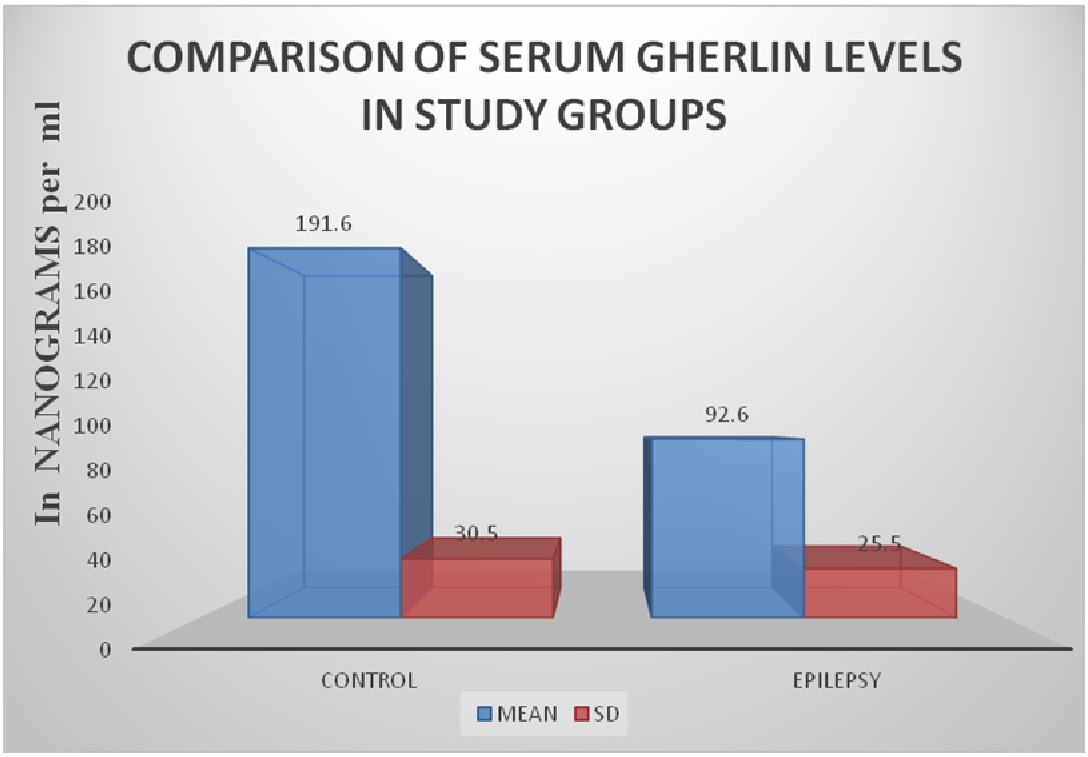
Variable	Subjects	Questionnaires	mean	SD	P – Value
Subjective sleep scores	Control	PSQI	0.77	1.33	0.000**
	GTCS	PSQI	4	2.69	
	Control	ESS	6.23	2.21	0.009*
	GTCS	ESS	8.56	4.22	
* P value < 0.001 very highly significant ** P – Value < 0.001 Very Highly Significant					

The difference between subjective sleep score in between study groups is statistically significant.

COMPARISON OF SERUM GHRELIN LEVELS

TABLE XIII: Comparison of serum ghrelin levels in between Control and GTCS patients

Variable	Group	N	Mean	SD	P –Value
Serum ghrelin(ng/ml)	Control	30	191.6	30.5	<0.000*
	GTCS	30	92.6	25.5	
* P – Value < 0.001 is very highly Significant					


COMPARISON OF SERUM GHERLIN LEVELS IN STUDY GROUPS
In NANOGRAMS per ml
200
180
160
140
120
100
80
60
40
20
0
191.6
30.5
92.6
25.5
CONTROL
EPILEPSY
MEAN
SD

DISCUSSION

This study was done to record sleep stages and events occurring during sleep and find the serum ghrelin levels in both generalized tonic clonic seizure patients and in control groups along with their subjective sleep scores. The mean age of the study groups were comparable 31.10±5.6 for GTCS patients and 30.03±6.26 for control groups as well as body mass index for GTCS patients (26.89±0.99) and for control groups (26.30±1.46). Both the group persons were subjected to polysomnography for a minimum period of 6 hours to maximum period of 8 hours. The obtained data were analyzed statistically and student't test was used to obtain the significance.

SCORING OF SLEEP STAGES:

Sleep stages were interpreted with respect to the duration of each sleep stage, sleep onset latency, total sleep duration, sleep efficiency. Total sleep time was recorded as the sum of all 30 second epochs of REM and non REM sleep. The mean total sleep time (NAP time) for GTCS patients 373.53±27.97 and for control group 374.08±28.15 minutes.

The mean sleep efficiency percentage of GTCS patients shows a very highly significant decrease 84.58±7.20 when compared to control group 91.58±3.66%. this may be the cause of sleep disruption in patients with epilepsy.

Sleep stage I of GTCS patients 18.35±2.85 minutes of mean value shows an highly significant increase when compared to control groups 14.6±2.6 minutes. Similar increase in first stage of sleep was obtained in the study done by (**Maganti R et al 2005**)[41]. Sleep stage II of GTCS patients 211.6±16.26 minutes of mean value shows a significant increase when compared to control groups 197.5±22 minutes. Excessive day time sleepiness is associated with increase in sleep stage I, increased arousal frequency leading to loss of continuity in sleep, decrease in duration of slow wave sleep and REM sleep.

Sleep stage III of GTCS shows a significant decrease 36.98±7.40 when compared to control group 40.9±7.2 minutes. Sleep stage IV of GTCS patients shows a decrease 42.65±9.22 but not a significant when compared to control group, 43.9±11 minutes.

Rapid eye movement sleep stage of GTCS patients shows a very highly significant decrease 63.93±13.21 when compared to control groups 77.27±13.3 minutes. Similar results were obtained in the study done by (**Maganti R et al 2005**).[41]

NAP onset latency is defined as the duration of time between lights out and sleep onset, defined by consecutive epochs of stage I or first stage of deeper NREM stage. The NAP onset latency measured in minutes for GTCS 15.52±5.9 shows an increase but not significant, when compared control groups 14.37±5.9 minutes.

Baldy-Moulini et al [72], conducted a study, childhood absence epilepsy, described several changes in sleep architecture including increased sleep latency, REM latency, REM percentage, and increased awakenings.

The mean percentage value of the sleep stages showed reduction in deep slow wave sleep and rapid eye movement stage, there by occupying the counter portion by the stage I & II as the following; Sleep stage I GTCS patients 4.93±0.68%, control group 3.90±0.66%, Sleep stage II GTCS patients 56.73±3.13%, control group 52.74±4.12%, Sleep stage III GTCS patients 9.88±1.90%, control group 10.97±1.88%, Sleep stage IV GTCS patients

11.45±2.51%, control group 11.73±2.61%, stage REM GTCS patients 17.02±2.78%, control group 20.66±3.20%. The slow wave sleep is reduced and the part is occupied by stage 1&2 of sleep.

Epileptiform discharges may themselves disrupt the quality of sleep and result in a chronically sleep-deprived state. The epileptiform discharges were primarily distributed in stages 1 and 2 of sleep, whereas they were rarely present during wakefulness and slow-wave sleep, and scoring in slow-wave sleep was technically difficult. Sleep deprivation resulting from such disruption in either the short term or the long term can facilitate seizures and epileptiform discharges (**Maganti R et al 2005**)[41].

Sleep architecture changes were, however, more significant when patients had nocturnal seizures compared with those who had no seizures (**Besset A et al) [73].**

PSG studies of adults with focal epilepsy showed mixed results. In temporal lobe epilepsy, for example, reduced sleep efficiency and REM sleep and increased wakefulness after sleep onset and lighter stages of sleep were found, especially with diurnal and nocturnal seizures (**Baldy-Moulinier et al**[74] and **Bazil.cw et al**[75]).

In frontal lobe epilepsy, reduced sleep efficiency and increased ratio of cyclic alternating pattern duration to non-REM sleep was noted (**Crespel A et al**[76] and **Zucconi et al**[77]**).**

Others have shown that sleep architecture can be abnormal in patients with focal epilepsy, even in the absence of clinical seizures, because interictal discharges can themselves cause repeated arousals.[78,79]Cortx F et al and Dahire et al.

Subjective sleep scores for GTCS patients 4±2.69 for PSQI were higher than for the control group 0.77±1.33, as well as higher scores for ESS obtained for shift worker 8.56±4.22 as compared to control group 6.23±2.21, showing poor quality of sleep for GTCS patients and more prone for dozing during the period when he or she usually is awake.

In my study, Serum ghrelin levels in GTCS patients shows a highly significant decrease 92.6±25.5 ng/ml, when compared with control group 191.6±30.5 ng/ml. These results were consistent with the study by **Z.Ataie et al 2010**[42].

These results also consistent with, studies conducted by **(Greco et al 2005**[43]**, Prodam et al 2010)** Circulating ghrelin levels were significantly lower in epileptic patients treated with valproate in pubertal and pre pubertal periods.

Aydin. S et al 2009[44] and **Dag E et al 2010**[45] conducted studies shows that both acylated and deacylated forms of serum and salivary ghrelin were depressed in epileptic patients before and after treatment with respect to normal controls.

Contradictorily, **Berilgen M.S et al 2006** demonstrated that ghrelin levels were higher in epilepsy patients who received antiepileptic drug therapy than a control group. These results also demonstrated by, **Gungor.S et al 2007**[46] and **Tomoum.H.Y et al 2009**[47] in children treated with valproate in comparison with healthy controls.

Z. Ataie et al 2011 conducted a study, its results shows ghrelin levels reduced following seizures in rats.

Two possibilities may be considered for this lowered ghrelin levels after seizures.

1. Uptake of Acylated ghrelin is increased by central nervous system structures to modulate epileptic discharges.
2. A feedback system reduces the ghrelin levels after seizures.

   In this feedback regulating mechanism, some hormones are involved in this action includes Somatostatin and Leptin.

Somatostatin is a peptide produced in the brain, pancreas, gastrointestinal system (**Shimada et al 2003**)[48] .It is released mainly from neurons under conditions of elevated activity such as seizures (**Vezzani, A et al 1999**)[49]. A single injection of somatostatin reduces the concentration of ghrelin in rats. In addition, somatostatin decreases GOAT expression, enzyme responsible for acylation of ghrelin (**Gahete, M.D et al 2010**)[50]. Therefore, it is possible that blood acylated ghrelin reduction is due to somatostatin released from one of its source following seizures.

The second possibility for this reduced ghrelin levels is high serum leptin levels following seizures, demonstrated in rats by **Bhatt, R et al 20005[51] and Hum, K.M et al 2009[52].** In this study results shows that, approximately leptin levels increased as twice as controls **(Hum, K.M et al 2009[52]). On other hand**, ghrelin is negatively regulated by leptin (**Asakawa, A et al 2001[53])** and physiological concentrations of leptin and hyperleptinemia directly inhibit the ghrelin secretion from the rat stomach (**Kamegai, J et al 2004[54])**. Therefore ghrelin reduction might be due to release of leptin following PTZ induced seizures.

Another possibility for this decreased ghrelin levels are human ghrelin exhibits saturable binding and endocytosis in the RBE4 rat cerebral microvessel endothelial cell line (**Pan, W et al**

2006)[55]. And blood brain barrier mechanisms regulating ghrelin accumulation by brain may be influenced by pathophysiological events. It has also been shown that ghrelin and its agonists can be considered as an antiepileptic agent in rodents (). Therefore reduction of this ghrelin levels followed by PTZ induced seizure could be attributed to its uptake by brain to represent an anti-epileptic effect.

One more possibility for this reduced ghrelin following seizures are degradation and proteolysis of acylated ghrelin into non-Unacylated ghrelin metabolites could be occurred during seizures (**De V riese, C et al 2004)[56].**

M. Said Berilgen et al 2005[57], conducted a study and his results shows that serum ghrelin levels were enhanced following a seizure episode. Cause of this elevated ghrelin after the seizures may be due to changes in growth hormone and prolactin levels after seizures and there by disrupting equilibrium of hormones, may facilitate the occurrence of seizures.

In humans, ghrelin act as an important factor in the sleep regulation.

A study conducted by **Weikel, J.C et al 2003**[58], its result shows that after an injection of ghrelin to normal healthy males, their slow wave sleep increased during night leads to non-REM sleep increased overall during the nigh. Stages of sleep are related to patterns of sleep occurrence. Epileptiform discharges multiply during non REM sleep stage. It is characterized by background of synchronized cellular discharges and reduced tone, which are also a sign of decreased wakefulness. In contrast, REM sleep resists the generation of epileptiform discharges (**Shouse, M.N et al 2002)**[59]**.**

Ghrelin secretion in healthy, non obese persons shows a diurnal course and that during night its level elevated **(Vaughn, B.V et al 2004**) [60]

According to this study, ghrelin has a capacity to prolong the NREM sleep –the stage in which seizures occur, and also this elevated ghrelin level in these epileptic patients be interpreted as a contributing factor in the occurrence of seizure. Ghrelin levels elevated more in partial epilepsy than generalized epilepsy. The reason for these elevation may be due to most localized seizures originated from the temporolimbic structures that include amygdala and hippocampus**(Falconer, M.A et al 2964)**[61]**.** Epileptiform discharges causes dysfunction in the temporolimbic region has

been shown to disrupt the hypothalamo pituitary regulation **(Herzog, A.G et al 1989 [62]and Bilo. L, et al 1991 [63])**.This suggests that higher ghrelin levels were observed in these patients with partial epilepsy are mediated by the effects of epileptiform discharges on the regulation of ghrelin by hypothalamus.

The use of antiepileptic drugs in this study patients, also may contribute to this elevated ghrelin levels.

The capacity of epilepsy to impair regulation in the hypothalamo pituitary axis independent of medication has been reported **(Zobel, A et al 2004)[64]**. Based on this study suggest that, ghrelin capacity to affect sleep stages and neuroendocrine physiology, both of which are linked to epilepsy, it may be suggested that higher ghrelin levels found in these patients indicate the predisposition towards seizures.

EFFECT OF AED's IN SLEEP ARCHITECTURE:

Although abnormalities in sleep architecture might be caused by anti epileptic drugs (AEDs), the results of studies are mixed. PHT, CBZ, Gabapentin (GBP), and Lamotrigine (LTG) have all been shown to affect sleep architecture (**Placidi F et al)[80],** although

others have shown that AEDs improve sleep quality by reducing the interictal discharges and EEG arousals (**Sammarilano M et al)**[81]**.**

Excessive daytime sleepiness was documented in de novo patients with epilepsy before AEDs were initiated and after sustained discontinuation (**Wolf P et al)**[82]**.**

Majority of patients Data on effects of VPA on sleep structure are not consistent. **VPA is known to have some hypnotic properties**; it has been shown not to alter the sleep architecture itself by some researchers (**Placidi F et al**)[80], whereas other studies have shown that it may reduce REM sleep (**Lagros B et al)**[83]or increase stage 1 sleep **(Palm L et al)**[84]**.**

Sleep architecture can be abnormal in children with epilepsy, an abnormality that may be independent of AED effect. It also is possible that the behavioural abnormalities may have been due to AEDs as well.

Several studies have shown that behavioural effects may be caused by a variety of AEDs, but literature in this area is confounded by inconsistent and contradictory results. Therefore no consistent conclusions can be made in this regard (**Blaise B et al)**[85]**.**

A study of children with newly diagnosed idiopathic generalized epilepsy before and after treatment may answer this question, but the study would be confounded by behavioral and attentional abnormalities caused by untreated epilepsy, and therefore methodologically difficult.

CONCLUSION

The following conclusions have been derived from the study

- Sleep efficiency percentage is significantly reduced in GTCS patients.
- Stage I and II sleep duration and percentage significantly increased signifying increased duration of light sleep and more propensity for arousal and sleep disruption in GTCS patients.
- There is significant decrease in percentage of REM sleep duration in GTCS patients. The lack of adequate deep sleep stages might be the underlying reason for the individual to wake up unrefreshed after his nocturnal sleep period.
- In conclusion, results obtained from my study showed that serum ghrelin level significantly reduced in GTCS patients than comparative with control group.
- Sleep architecture is abnormal in patients with generalized epilepsy. Further studies are needed to

determine whether abnormalities in sleep architecture contribute to poor daytime behaviour and attention.

- In the future, a better understanding of the mechanisms responsible for seizure – induced alterations in hormone levels of plasma may help to prevent these changes.

LIMITATIONS

- The main limitation of my study is that we were unable to control the medication effects.
- Sample size is an important limiting factor in this study.
- Only few numbers of prior studies available. Ghrelin hormone is still under in research..
- Associated sleep disorders, it may also creates changes in the sleep architecture.

SUMMARY

- This study was conducted to assess sleep and the sleep parameters in generalized tonic clonic patients in comparison with control group.

- Thirty GTCS patients and thirty healthy control patients participated in the study. The results were statistically analyzed and tabulated for discussion.

- Serum ghrelin levels were decreased significantly in these patients than compared with control group.

- It was found that generalized tonic- clonic seizure patients have changes in the sleep pattern in the form of prolonged stage I and stage II of sleep with reduced REM sleep duration and sleep efficiency leads to fragmentation of sleep. Prolonged non REM sleep is an important precipitating factor for seizure propagation.

- Due to the complex relationship between the epilepsy and sleep disorders, these sleep changes must be addressed in order to provide the best management of sleep disturbance in patients with epilepsy.

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INFORMED CONSENT FORM

Title of the study: "A study of Polysomnography and serum Ghrelin levels in patients with generalized tonic-clonic seizures".

Name of the Participant:

Name of the Principal Investigator: Dr. K. Meenakumari

Name of the Institution:

Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"A study of Polysomnography and serum Ghrelin levels in patients with Generalized tonic clonic seizures.

1) I have read and understood this consent form and the information provided to me.

2) I have had the consent document explained to me.

3) I have been explained about the nature of the study.

4) I have been explained about my rights and responsibilities by the investigator.

5) I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

6) I have been advised about the risks associated with my participation in this study.

7) I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

8) I have not participated in any research study within the past _____month(s).

9) I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

10) I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

11) I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

12) I have understood that my identity will be kept confidential if my data are publicly presented.

13) I have had my questions answered to my satisfaction.

14) I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly

explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____

Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____

Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____

Signature_____

Date_____

PROFORMA

1) Name :

2) Age:

3) Sex:

4) Address :

5) Occupation :

6) Complaints/duration:

7) History of present illness:

8) History of any sleep disturbance after the onset of epilepsy?

9) Past history:

10) History of any drug intake

11) History of associated illness:

    a. Diabetes

    b. Hypertension

    c. Ischemic heart disease

    d. Respiratory diseases

    e. Renal diseases

INVESTIGATIONS :

Fasting serum Ghrelin level

Polysomnography

EXAMINATION:

General examination:

Temperature:

Pulse rate

Blood pressure:

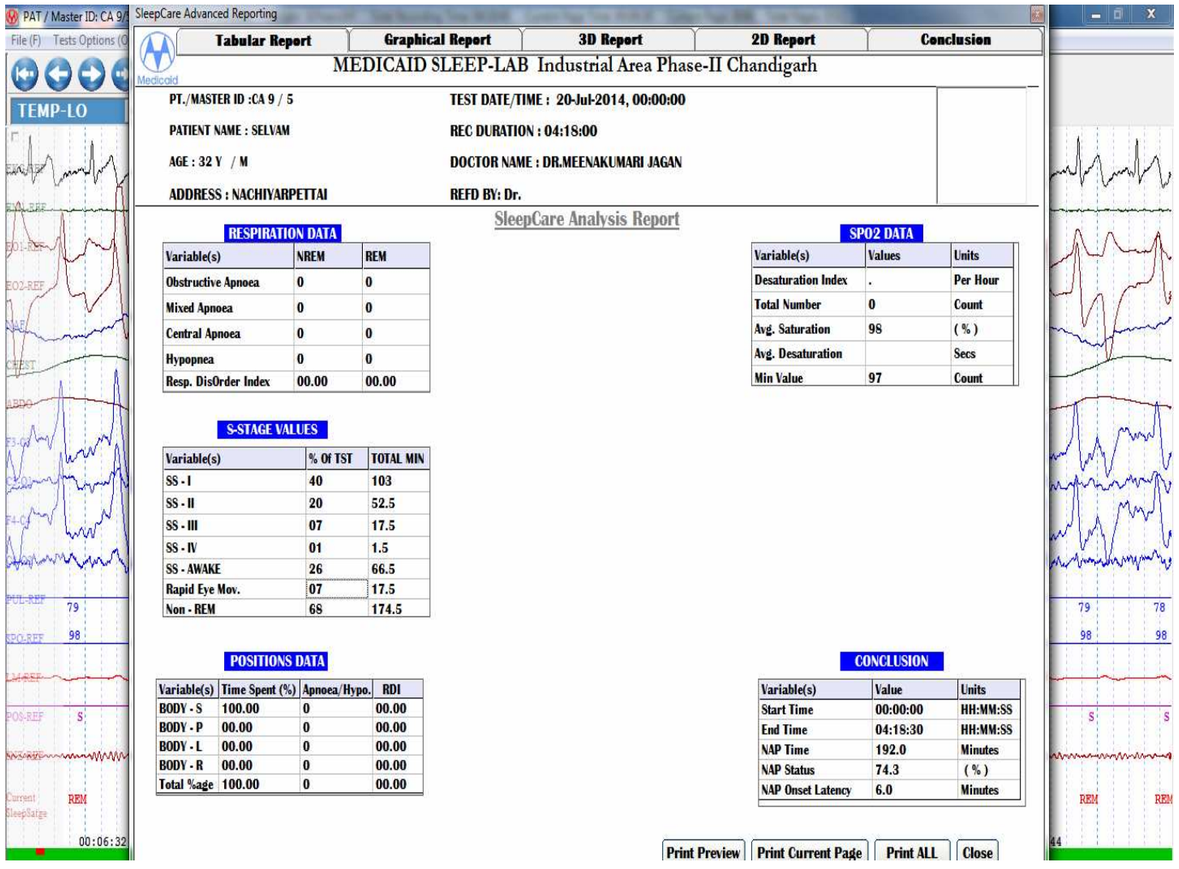
Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:


SleepCare Advanced Reporting
Tabular Report
Graphical Report
3D Report
2D Report
Conclusion
MEDICAID SLEEP-LAB Industrial Area Phase-II Chandigarh
PT./MASTER ID :CA 9 / 5
TEST DATE/TIME : 20-Jul-2014, 00:00:00
PATIENT NAME : SELVAM
REC DURATION : 04:18:00
AGE : 32 Y / M
DOCTOR NAME : DR.MEENAKUMARI JAGAN
ADDRESS : NACHIVARPETTAI
REFD BY: Dr.
SleepCare Analysis Report
RESPIRATION DATA
Variable(s) NREM REM
Obstructive Apnoea 0 0
Mixed Apnoea 0 0
Central Apnoea 0 0
Hypopnea 0 0
Resp. DisOrder Index 00.00 00.00
SPO2 DATA
Variable(s) Values Units
Desaturation Index . Per Hour
Total Number 0 Count
Avg. Saturation 98 (%)
Avg. Desaturation Secs
Min Value 97 Count
S-STAGE VALUES
Variable(s) % Of TST TOTAL MIN
SS - I 40 103
SS - II 20 52.5
SS - III 07 17.5
SS - IV 01 1.5
SS - AWAKE 26 66.5
Rapid Eye Mov. 07 17.5
Non - REM 68 174.5
POSITIONS DATA
Variable(s) Time Spent (%) Apnoea/Hypo. RDI
BODY - S 100.00 0 00.00
BODY - P 00.00 0 00.00
BODY - L 00.00 0 00.00
BODY - R 00.00 0 00.00
Total %age 100.00 0 00.00
CONCLUSION
Variable(s) Value Units
Start Time 00:00:00 HH:MM:SS
End Time 04:18:30 HH:MM:SS
NAP Time 192.0 Minutes
NAP Status 74.3 (%)
NAP Onset Latency 6.0 Minutes
Print Preview
Print Current Page
Print ALL
Close
TEMP-LO
REM
00:06:32

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு :

பாலிசோம்னோகிரா∴பி மற்றும் இரத்த கிரெலின் அளவை வலிப்பு நோயாளிகளிடம் ஆராய்ந்து அறிதல்

பெயர் :

வயது:

பாலினம் : ஆண்/பெண்

நோயாளி அடையாள எண் :

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

எனது பாலிசோம்னோகிரா∴பி மற்றும் இரத்த கிரெலின் அளவை பரிசோதனை செய்ய முழு சம்மதம்.

இந்த ஆராய்ச்சியில் யாருடைய நிர்பந்தமுமின்றி சொந்த விருப்பத்தின் பேரில் சம்மதிக்கிறேன்.

இந்த ஆராய்ச்சியில் இருந்து நான் எந்த நேரமும் பின் வாங்கலாம் என்றும், அதனால் எந்த பாதிப்பும் எற்படாது என்பதையும் புரிந்து கொண்டேன்.

நான் என் வலிப்பு நோய் குறித்த இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல்களை பெற்றுக்கொண்டேன்.

இர்த்த கிரெலின் பரிசோதனைக்கு எனக்கு ஊசி மூலம் இரத்தம் எடுக்க சம்மதிக்கிறேன். மேற்கண்ட ஊசியை செலுத்தி இரத்தம் எடுக்கும் போது வலி, அரிப்பு, மயக்கம், போன்ற பின் விளைவுகள் எற்படல்லம் என்று தெரிந்து கொண்டேன்.

நான் என்னுடைய சுய நினைவுடன் மற்றும் முழு சம்மதத்துடன் இந்த ஆராய்ச்சிக்கு என்னை பரிசோதிக்க சம்மதிக்கிறேன்.

பங்கேற்ப்பாளர் கையொப்பம்

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.K.Meenakumari,
Postgraduate
Institute of Physiology and Experimental Medicine,
Madras Medical College, Chennai-3.

Dear **Dr.K.Meenakumari,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"A study of Polysomnography and serum Ghrelin levels in patients with generalized tonic clonic seizures"** No.14042014

The following members of Ethics Committee were present in the meeting held on 11.03.2014 conducted at Madras Medical College, Chennai-3.

1. Dr. C.Rajendran, M.D, -- Chairperson
2. Prof. Kalaiselvi, M.D, -- Member Secretary
   Vice Principal, MMC, Ch-3
3. Prof. Nandhini, M.D, -- Member
   Inst. of Pharmacology, MMC, Ch-3
4. Prof.Bhavani Sankar, M.S, -- Member
   Prof & HOD General Surgery, MMC, Ch-3
5. Prof.V.Padmavathi, M.D, -- Member
   I/c. Director of Pathology, MMC, Ch-3
6. Thiru. S. Govindasamy, BA., BL -- Lawyer
7. Tmt.Arnold Saulina, MA MSW -- Social Scientist
8. Thiru.S.Ramesh Kumar, -- Lay Person
   Administrative Officer, MMC, Ch-3.

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

15/4/14

						Master Chart - Control group												
S NO:	SS -I	SS -II	SS -III	SS -IV	REM	Non- REM	SS -I	SS -II	SS -III	SS -IV	Rapid Eye	Non- REM	NAP time	NAP status	NAP onset	ghrelin pg/mi	PSQI	ESS
1	8	125	40	45.5	50.5	218.5	2.97%	46.47%	14.87%	16.91%	18.77%	81.23%	269	91.5	5	169	0	13
2	17	201	40	65.5	75	323.5	4.27%	50.44%	10.04%	16.44%	18.82%	81.18%	398.5	92.25	12.5	98	0	3
3	17	220	60.5	80	53	377.5	3.95%	51.10%	14.05%	18.58%	12.31%	87.69%	430.5	89.97	14	89	1	7
4	11	180	45	52	62	288	3.14%	51.43%	12.86%	14.86%	17.71%	82.29%	350	75.57	18	78	1	4
5	17	179	35	35	67	266	5.11%	53.75%	10.51%	10.51%	20.12%	79.88%	333	88.03	20.5	178	5	8
6	13.5	181.5	45	43	91	283	3.61%	48.53%	12.03%	11.50%	24.33%	75.67%	374	92.52	7.5	56	5	7
7	18	165	44	9	85	283	4.89%	44.84%	11.96%	15.22%	23.10%	76.90%	368	89.64	14	79.8	0	6
8	12.5	176.5	46	50	93.5	285	3.30%	46.63%	12.15%	13.21%	24.70%	75.30%	378.5	90.39	6	120	0	6
9	15.5	178.5	43.5	55	84.5	292.5	4.11%	47.35%	11.54%	14.59%	22.41%	77.59%	377	95.8	14	110.5	0	8
10	15	189.5	44.5	34.5	68.5	283.5	4.26%	53.84%	12.64%	9.80%	19.46%	80.54%	352	95.67	12	124.5	0	8
11	16.5	215.5	54	45	63	331	4.19%	54.70%	13.71%	11.42%	15.99%	84.01%	394	94.08	6.5	100.6	2	8
12	10.5	223.5	42	32.5	65.5	308.5	2.81%	59.76%	11.23%	8.69%	17.51%	82.49%	374	90.12	8.5	98.6	0	7
13	12	213	40	40	65.5	305	3.24%	57.49%	10.80%	10.80%	17.68%	82.32%	370.5	91.12	18	157	2	4
14	11.5	202	36	39	70.5	288.5	3.20%	56.27%	10.03%	10.86%	19.64%	80.36%	359	93.24	28	90.9	0	6
15	15.5	198.5	31	45	102	290	3.95%	50.64%	7.91%	11.48%	26.02%	73.98%	392	91.65	24	120.3	2	4
16	16	201	38	43	83.5	298	4.19%	52.69%	9.96%	11.27%	21.89%	78.11%	381.5	93.45	15	111	0	5
17	16.5	172	43	39	91	270.5	4.56%	47.58%	11.89%	10.79%	25.17%	74.83%	361.5	94.63	19	99	0	3
18	12.5	203	28	34	87	277.5	3.43%	55.69%	7.68%	9.33%	23.87%	76.13%	364.5	91.35	9.5	145	0	4
19	17	182	48	45	91	292	4.44%	47.52%	12.53%	11.75%	23.76%	76.24%	383	91.72	6	174.5	0	9
20	14.5	216.5	38	26	90.5	295	3.76%	56.16%	9.86%	6.74%	23.48%	76.52%	385.5	95.66	10	90.7	0	6
21	17.5	202	31	43	70.5	293.5	4.81%	55.49%	8.52%	11.81%	19.37%	80.63%	364	90.32	16.5	123	0	4
22	16.5	219	35	39	58	309.5	4.49%	59.59%	9.52%	10.61%	15.78%	84.22%	367.5	92.79	12	167	1	8
23	12.5	212	36	32	75	292.5	3.40%	57.69%	9.80%	8.71%	20.41%	79.59%	367.5	93.75	18	90.5	0	5
24	17	210	33	41	68	301	4.61%	56.91%	8.94%	11.11%	18.43%	81.57%	369	95.84	22	143	1	8
25	16	232	38	48	75	334	3.91%	56.72%	9.29%	11.74%	18.34%	81.66%	409	88.53	24.5	104.9	0	6
26	14.5	199	49	40	82	302.5	3.77%	51.76%	12.74%	10.40%	21.33%	78.67%	384.5	90.6	17	120	0	4
27	12.5	224	45	42.5	91.5	324	3.01%	53.91%	10.83%	10.23%	22.02%	77.98%	415.5	92.61	13.5	110	0	6
28	11	218	34	49	86.5	312	2.76%	54.71%	8.53%	12.30%	21.71%	78.29%	398.5	89.55	17	110.5	1	4
29	16.5	200	36	35.5	90	288	4.37%	52.91%	9.52%	9.39%	23.81%	76.19%	378	94.1	10.5	99	1	9
30	17	185	49	40	82	291	4.56%	49.60%	13.14%	10.72%	21.98%	78.02%	373	91.09	12	90	1	7

						Master Chart - GTCS group												
S NO:	SS -I	SS -II	SS -III	SS -IV	REM	Non- REM	SS -I	SS -II	SS -III	SS -IV	Rapid Eye	Non- REM	NAP time	NAP status	NAP onset	ghrelin pg/ml	PSQI	ESS
1	15.5	200	15	35.5	45	266	4.98%	64.31%	4.82%	11.41%	14.47%	85.53%	311	68	5	56.4	1	2
2	15.5	167.5	20.5	46	42.5	249.5	5.31%		7.02%	15.75%	14.55%	85.45%	292	80.66	12.5	46.8	3	5
3	15.5	185.5	45	40	43	286	4.71%	56.38%	13.68%	12.16%	13.07%	86.93%	329	90.07	14	67.5	7	11
4	19.5	198	38	49	52	304.5	5.47%	55.54%	10.66%	13.74%	14.59%	85.41%	356.5	74.57	18	70.6	3	13
5	19.5	224	28.5	33	50.5	305	5.49%	63.01%	8.02%	9.28%	14.21%	85.79%	355.5	91.28	22	67	4	1
6	15.5	205	37	40	81	297.5	4.10%	54.16%	9.78%	10.57%	21.40%	78.60%	378.5	89.2	7.5	88	6	1
7	21	197	41	54	50	313	5.79%	54.27%	11.29%	14.88%	13.77%	86.23%	363	86.95	14	65.9	2	11
8	14.5	202.5	43	42	77.5	302	3.82%	53.36%	11.33%	11.07%	20.42%	79.58%	379.5	82.41	12	55	4	4
9	17.5	200.5	44.5	44	44	306.5	4.99%	57.20%	12.70%	12.55%	12.55%	87.45%	350.5	89.5	14	66.9	4	12

10	16.5	185.5	40	45.5	55	287.5	4.82%	54.16%	11.68%	13.28%	16.06%	83.94%	342.5	92.13	7	87	7	6
11	17	220	29	46.5	60.5	312.5	4.56%	58.98%	7.77%	12.47%	16.22%	83.78%	373	90.03	9.5	76.9	4	10
12	20.5	208.5	39	40.5	75	308.5	5.35%	54.37%	10.17%	10.56%	19.56%	80.44%	383.5	86.07	8.5	77	2	12
13	17.5	220	45	46	52.5	328.5	4.59%	57.74%	11.81%	12.07%	13.78%	86.22%	381	86.79	20	39.7	3	10
14	13.5	200.5	39.5	47.5	60.5	301	3.73%	55.46%	10.93%	13.14%	16.74%	83.26%	361.5	90.07	28	50.6	13	9
15	17.5	220.5	40	46.5	88	324.5	4.24%	53.45%	9.70%	11.27%	21.33%	78.67%	412.5	89.01	24	54.6	4	12
16	19.5	230.5	38.5	40	62.5	328.5	4.99%	58.95%	9.85%	10.23%	15.98%	84.02%	391	85.5	19.5	76	3	11
17	20	200.5	40.5	45.5	82	306.5	5.15%	51.61%	10.42%	11.71%	21.11%	78.89%	388.5	89.67	19	80	2	11
18	25	236.5	25.5	44	76	331	6.14%	58.11%	6.27%	10.81%	18.67%	81.33%	407	80.57	9.5	49.6	8	9
19	20	212.5	45	57	60	334.5	5.07%	53.87%	11.41%	14.45%	15.21%	84.79%	394.5	75.61	10	50.4	6	9
20	18.5	219.5	35	3.55	79.5	276.55	5.20%	61.65%	9.83%	1.00%	22.33%	77.67%	356.05	67.63	18	90	3	15
21	20.5	214.5	33.5	40	55.5	308.5	5.63%	58.93%	9.20%	10.99%	15.25%	84.75%	364	86.75	16.5	78.8	1	11
22	19.5	223	38.5	46.5	60	327.5	5.03%	57.55%	9.94%	12.00%	15.48%	84.52%	387.5	87.79	12	56	4	10
23	24	235.5	33.5	30	64	323	6.20%	60.85%	8.66%	7.75%	16.54%	83.46%	387	84.66	18	70	4	7
24	16.5	214	45.5	48.5	66	324.5	4.23%	54.80%	11.65%	12.42%	16.90%	83.10%	390.5	88.86	22	49	2	10
25	21.5	235	35.5	45	64	337	5.36%	58.60%	8.85%	11.22%	15.96%	84.04%	401	79.88	26	76.5	8	12
26	17.5	222	45	46.5	67.5	331	4.39%	55.71%	11.29%	11.67%	16.94%	83.06%	398.5	67.99	20	47.6	3	12
27	17.5	226.5	35	38.5	70	317.5	4.52%	58.45%	9.03%	9.94%	18.06%	81.94%	387.5	85.86	15.5	89	4	13
28	14.5	222	40	45.5	75.5	322	3.65%	55.85%	10.06%	11.45%	18.99%	81.01%	397.5	92.19	21	60	2	7
29	17	220.5	32.5	45	79	315	4.31%	55.96%	8.25%	11.42%	20.05%	79.95%	394	88.84	10.5	46.8	2	2
30	22.5	200.5	41	48	79.5	312	5.75%	51.21%	10.47%	12.26%	20.31%	79.69%	391.5	89	12	98	1	2